

Editorial

The Year 2024, Nigeria's Physiology Family and the Plethora of Exits

Olaleye S.B.

Department of Physiology, College of Medicine, University of Ibadan. Ibadan, Nigeria

Summary: The Physiological Society of Nigeria mourns the loss, in the first half of the year 2024, of five distinguished members who left indelible marks and footprints in the sand of time. The contributions of Professors Chikodi Anigbogu (23 December 1953 – 2 January 2024); David Oyebola (1 October 1945 - 13 February 2024); Anthony Ebeigbe (22 August 1950 – 30 May 2024); Vincent Iyawé (14 April 1953 – 8 June 2024) and Olusoga Solofa (24 January 1947 – 10 June 2024) to the establishment and growth of the Physiological Society of Nigeria are well appreciated.

Keywords: Tribute, Physiological Society of Nigeria, Nigerian physiologists, Anigbogu, Oyebola, Ebeigbe, Iyawé, Solofa

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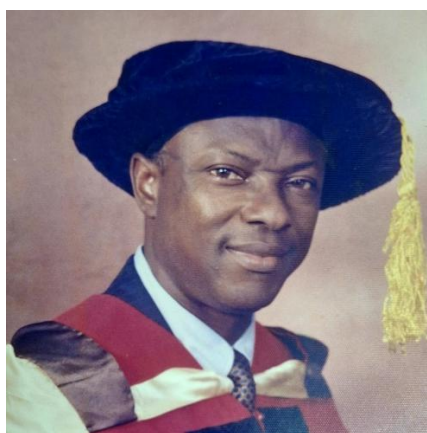
The Physiology family in Nigeria woke up on the first day of the year 2024 with traditional greetings and best wishes from many of her highly respected elders. One of the earliest messages I received came from Prof. Chikodi Nnanyelu Ezegwui Anigbogu, whose retirement from the University of Lagos was celebrated at a well-attended senate meeting on Thursday December 14, 2023. It was thus a great shock when the news of the death of Prof. Anigbogu was announced on the 2nd of January 2024. He had been a very strong pillar in the society since its establishment in 1978, having held positions such as Secretary general (1991 – 1994), Treasurer and International Liaison Officer of the society.

Born on December 23, 1953, in Enugu, Enugu State, Nigeria and being an alumnus of the University of Ibadan, Nigeria (B.Sc. Physiology class of 1979), 'Gbogus' as he was fondly called by his peers, obtained M.Sc. and Ph.D. degrees from the University of Lagos, Nigeria in 1983 and 1993 respectively. He

will be remembered for his love for the growth of the Physiological Society of Nigeria and his penchant for suggesting avenues for peaceful resolution of conflicts within the society. He supervised ten Ph.Ds, several M.Sc. and B.Sc students in his over 43 year career in the university system,

While still brooding on the unexpected loss of Prof Anigbogu, members of the Society gathered in Port Harcourt from Tuesday, February 13, 2024, to attend the 41st Annual Scientific meeting of PSN. The sad news of the death of one of the founding fathers of the society filtered in. Prof. David Dada Oyewusi Oyebola was reported to have died in the early hours of Tuesday 13th February 2024 after a brief illness.

Prof. Oyebola attended the University of Ibadan medical school between 1965 and 1972. Throughout his undergraduate years, he enjoyed scholarship made on merit to outstanding candidates in each faculty in the concessional entrance examination to the University of Ibadan.



Prof. C.N.E. Anigbogu, FPSN
23 December 1953 – 2 January 2024



Prof. D.D.O. Oyebola, FPSN
1 October 1945 - 13 February 2024



Prof. A.B. Ebeigbe, FPSN
22 August 1950 – 30 May 2024

As a result of his outstanding performance in the Part 1 MBBS examination in March 1968, he was invited to do an extra year for the intercalated B.Sc. Med. Sci. degree in Physiology and graduated with a first class Honours degree in the B.Sc programme in June 1969. He was also a part-time demonstrator in Physiology from 1968 to 1973. He eventually obtained the M.B.B.S degree in June, 1972.

Prof. Oyebola was a foremost teacher, mentor, former head of department of Physiology, University of Ibadan, former Dean, Faculty of Basic Medical Sciences, University of Ibadan, Foundation provost, College of Medicine, Ekiti State University, Foundation Editor of the Nigerian Journal of Physiological Sciences, former President of the Physiological Society of Nigeria and former Council member of International Union of Physiological Sciences. After teaching Physiology for 41 years, Prof. Oyebola retired from the University after a highly successful and meritorious service in September 2011. He was one of the first set of elected fellows of the Physiological Society of Nigeria in 2010. Prof. Oyebola, an esteemed scholar and prolific author, will be remembered for his hard work, passion and immense contribution to physiology education and practice.

One of the elders who attended the 41st PSN conference in Port Harcourt was Prof. Anthony Ebeigbe, a man who served the society with all his vigour. Except when not in the country, Prof Ebeigbe was known to be a sure attendee of PSN conferences, giving talks to encourage upcoming physiologists, offering counsels on issues requiring attention of the executive and relating with colleagues in a way that endeared him to all generations of physiologists. We left Port Harcourt with plans for him to be actively involved in the burial program of Prof. D.D.O. Oyebola, unknown to us that the Port Harcourt conference was going to be his last major outing. He fell ill soon after the conference and the news of his death on Thursday 30th May 2024 was a rude shock to many of us.

Prof Ebeigbe was trained at Nigeria's premier University (University of Ibadan), graduating with a degree in Physiology in 1974. He was a foundation staff of University of Benin in 1975 as a Graduate Assistant and furthered his pursuit of a higher degree at the Institute of Physiology at the University of Glasgow in Scotland, from where he got a doctoral degree in 1980. He returned to Nigeria and rose through the ranks to become an Associate Professor of Physiology in 1984. In 1990, he was appointed Professor of Physiology at the Bendel/Edo State University (now Ambrose Alli University), Ekpoma. At Ekpoma, he was Dean, Faculty of Basic Medical Sciences between 1991 and 1993. He later returned to base at the University of Benin as professor (by

appointment) in 1993. He was elected Provost, College of Health Sciences, University of Benin between 1993 and 1994. He also served as the acting Vice Chancellor of PAMO University of Medical Sciences, Port Harcourt, on its establishment in 2018.

Prof Ebeigbe had research exposures in several laboratories around the world. He was at the Physiology Department, University of Michigan Medical School, USA as a visiting Research Scientist and later as a Fulbright Scholar in 1982. In 1985, he was Research fellow at the Preclinical Research laboratories of Sandoz Ltd., Basel, Switzerland. Between 1987 and 2005, he was visiting Professor to Universities and Pharmaceutical companies in Brussels (Belgium), Basel (Switzerland), Glasgow (U.K), London (U.K) and Newcastle (U.K). In 2004 – 2005, he was Richard A. Bernstein distinguished Professor at the Department of Natural Sciences, University of Maryland, Eastern Shore, Maryland, U.S.A. Prof Ebeigbe was a recipient of many research grants and awards both within and outside Nigeria.

Tony, as he was fondly called, was the Society's National Secretary between 1983 and 1987. He became the second Editor-in-Chief of the Nigerian Journal of Physiological Sciences in 1987, a position he held till 2001, when he became the Vice President. He is thus the longest serving Editor-in-Chief of the Journal till date. During his tenure as editor, he exposed the Nigerian Journal of Physiological Sciences to several University libraries around the globe. He also laid the foundation for the Journal's inclusion in Medline and many other Indexing services. Prof Ebeigbe became the President, Physiological Society of Nigeria in 2005 at the Society's meeting in Port Harcourt and served in that capacity till 2010. He was until his death, a member of both the Board of Trustees (B.O.T) and also the Chairman of the Body of Fellows (B.O.F), of the Physiological Society of Nigeria. His exit will surely be missed but the indelible marks he left can never be erased.

The demise of Prof. Vincent Imagbovwon Iyawe's occurred a week after the death of Prof. Ebeigbe, on June 8, 2024. Prof. Iyawe had retired from the University of Benin in April 2023 having served the University for over 40 years.

Vincent Iyawe was admitted to read pharmacology at the University of Ibadan in 1972 but left after a year to pursue a career in medicine and surgery at the University of Benin. Where he graduated in 1978. Before and after his retirement, Prof Iyawe occupied several key administrative positions at the University of Benin and Benson Idahosa University. He was Provost, of the College of Medical Sciences, University of Benin, and until his death Provost of the College of Medical Sciences at the Benson Idahosa University and Bishop at the Church of God Mission.

In addition to the above positions, he was a member of the Governing Councils of the University of Benin and Ambrose Alli University; a member of the Board of Management of the University of Benin Teaching Hospital and Irrua Specialist Teaching Hospital.



Prof. Vincent Iyawe, FPSN
(14 April 1953 – 8 June 2024)



Prof. Olusoga Adekunle Sofola, FPSN
(24 January 1947 – 10 June 2024)

The last of the exits recorded in June 2024 was that of another founding member of the Physiological Society of Nigeria, Prof. Olusoga Sofola, who until death was an emeritus professor of Physiology at the University of Lagos. Prof. Sofola was an embodiment of leadership and a great mentor to many who had the opportunity of knowing him. He was a gentle elder who listened to younger colleagues, always willing to help build the research potential of younger physiologists. Through his magnanimity, many young scientists benefitted from his collaborative prowess in research and enjoyed several academic exchange programmes in laboratories of his colleagues within and outside Africa.

Olusoga Adekunle Sofola was born in Lagos on 24th January 1947 and obtained BSc, MSc and MBBS degrees from the College of Medicine, University of Lagos and PhD at the Department of Cardiovascular Studies, University of Leeds, where he was a British

Heart Foundation fellow. He began a long teaching and professional career at the College of Medicine, University of Lagos in 1973, where he was Head of Department for many years and supervised many reputable physiologists who are promoting the subject of Physiology all over the world today. He also taught in many other countries, including United Arab Emirates, Centre for Cardiovascular Diseases, Texas Southern University, Houston, the also the United Kingdom.

He had significant appointments in his remarkable lifetime including the Provost of the College of Medicine of the University Lagos (CMUL) (1993 - 1996), Deputy Vice Chancellor (Academics), University of Lagos. Lagos, Nigeria (2003 - 2007). In 2009, he was appointed as Vice-Chancellor of Olabisi Onabanjo University (OOU), Ago Iwoye, Ogun State, Nigeria. He was also the Vice Chancellor, Eko University of Medical Sciences, Lagos.

Professor Sofola was a founding member and a Fellow of the Physiological Society of Nigeria. He was a past President of Association of African Physiological Sciences, AAPS and a member of Council of the International Union of Physiological Sciences (IUPS). He was also a Fellow and former Treasurer of Nigerian Academy of Science. It is on record that Prof. Sofola was a major facilitator of the hosting of the International Union of Physiological Sciences (IUPS) Education workshop which was held jointly with the 34th Annual Scientific Conference of the Physiological society of Nigeria at the Sheraton Hotel, Lagos, Nigeria between October 20 and 23, 2014. He was a guest speaker at the IUPS congress in Birmingham, UK, in 2013. He delivered by Olusoga Sofola titled 'Physiology: The laboratory, the Hospital and Society' at the 30th PSN conference on 13th February 2014. On Friday, 31st August 2018, he delivered the Horatio Oritsejomi Thomas Distinguished Fellowship Lecture with the title "Improvement in Patient Care Through Basic & Clinical Research: Whither Nigeria" at the Trenchard Hall, University of Ibadan.

Prof Sofola once identified the description and elucidation of the mechanisms of the cardiovascular responses in an experimental tetanus model as a major highlight of his research career. Also, alongside his colleagues at Leeds, he was able to report the switch/change in blood vessel / vascular endothelial function during salt loading in which they were the first to demonstrate that the vascular endothelium can use other molecular channels to respond during salt loading.

Although Professor Soga Sofola's sojourn in the land of the living has ended, majority of us who had the privilege of knowing him agree that his years on earth were extremely impactful.

ABSTRACTS OF THE PROCEEDINGS OF THE XLIST ANNUAL SCIENTIFIC CONFERENCE OF THE PHYSIOLOGICAL SOCIETY OF NIGERIA

13-16 February 2024, Rivers State University, Port Harcourt and PAMO University of Medical Sciences, Port Harcourt, Rivers State, Nigeria.

THE IMPACT OF *OCIMUM GRATISSIMUM* ON SOME FERTILITY HORMONES AND HISTOLOGY OF THE OVARY AND UTERUS IN FEMALE WISTAR RATS

***Akpe, P.E., Emozozo, O.A., and Ebomoyi, M.I.**

Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Nigeria.

Email: pauline.akpe@uniben.edu; Tel: +2348035124737

Ocimum gratissimum, popularly known as scent leaf, is one of the medicinal plants with the potential to serve as an alternative therapy for the treatment of various ailments or as a source of a new drug. The aim of this research was to determine the effect of *Ocimum gratissimum* leaf extract on some fertility hormones in female wistar rats. Twenty four adult female wistar rats were randomly divided into 4 groups (n=6) consisting of low dose (LD), medium dose (MD), and high dose (HD) groups which were orally administered with 100mg/kg, 300mg/kg, and 500mg/kg doses of *Ocimum gratissimum* leaf extract respectively, and the control (C) group which received normal feed and water for four weeks. Results showed that there were significant decreases in oestrogen levels for LD and HD groups compared with the control and a significant increase in progesterone level for MD group compared with control. Histological analysis of the ovarian samples showed increased folliculogenesis and active stromal congestion in the LD group while the MD and HD groups showed decreased folliculogenesis and active stromal congestion when compared to control. Uterine histology showed active stromal congestion and glandular cell epitheliosis for all groups when compared to control with the LD group showing endometrial lining epithelial crowding and cytoplasmic clearing and MD group showing endometrial lining epithelial hyperplasia. Conclusively, *Ocimum gratissimum* showed a decrease in estrogen level with an increased folliculogenesis at low dose and an increase in progesterone level with endometrial lining hyperplasia at medium dose in female wistar rats.

Keywords: *Ocimum gratissimum*, fertility hormones, contraceptive, folliculogenesis, stromal congestion, epithelial hyperplasia.

EFFECT OF CHRONIC CONSUMPTION OF CALABASH CHALK ON GASTRIC ULCER SCORE IN ALBINO WISTAR RATS.

Okpo-Ene A.I., A.O. Obembe E.E. Osim

Department of physiology, University of Calabar.

Calabash chalk is a naturally occurring substance that is readily consumed all over the world as a form of geophagy and pica (Dean et al, 2004). Its consumption has been known

to cause histomorphological changes to the architecture of the gastrointestinal tract (Ekong et al, 2009). This research was aimed to study its effects on gastric ulcer score and its predisposing factors. Forty (40) adult Albino Wistar rats were randomly divided into 4 groups of 10 rats and each group was randomly sub-divided into 2 groups of 5 rats each. Group 1 served as the test group and was administered 1ml of calabash chalk suspension (40mg/kg) while group 2 served as the control group and was administered 1ml of distilled water orally and daily for 28 days. The mean basal gastric acid output in the test group was $0.61 \pm 0.37 \mu\text{Mol}/10\text{min}$ while that of the control group was $0.58 \pm 0.22 \mu\text{Mol}/10\text{min}$. There was no significant difference in basal gastric acid output between the 2 groups. The pH value of the test group was 2.94 ± 0.14 while that of the control group was 4.10 ± 0.37 . The pH of the test group was significantly lower than that of the control group ($P < 0.05$). The mean gastric mucous output of the test group was $0.08 \pm 0.01\text{g}$ while that of the control group was $0.14 \pm 0.014\text{g}$. The gastric mucous output in the test group was significantly reduced ($P < 0.01$) when compared to the control. The mean gastric pepsin concentration in the test group was $0.57 \pm 0.02\text{mg}/100\text{ml}$ while that of the control group was $0.46 \pm 0.02 \text{mg}/100\text{ml}$. The gastric pepsin concentration in the test group was significantly higher ($P < 0.01$) when compared to the control group. The mean gastric ulcer score in the test and the control groups were 7.50 ± 1.25 and 3.70 ± 0.30 respectively. The ulcer score was significantly higher ($P < 0.05$) in the test group. In conclusion, chronic consumption of calabash chalk leads to low gastric pH, increased pepsin concentration and low gastric mucous which predisposed to a higher ulcer score in Albino Wistar rats.

AMELIORATIVE EFFECTS OF THE HYDROMETHANOL LEAF EXTRACT OF *CRATERISPERMUM SCHWEINFURTHI* ON PHENYL HYDRAZINE INDUCED ANEMIA IN MALE WISTAR RATS

***Saronee F¹, Amah-Tariah FS², Chinko BC³, Dapper DV³**

Department of Physiology, Faculty of Basic Medical Sciences, ¹PAMO University of Medical Sciences, ²Rivers State University, and ³University of Port-Harcourt, Nigeria Port-Harcourt, Port-Harcourt, Nigeria

Email: fsaronee@pums.edu.ng

Anaemia is a clinical condition characterized by a reduction in the normal values of haemoglobin concentration, circulating erythrocytes and other erythrocyte indices per unit of blood. The present study evaluates the potential ameliorative effects of the hydromethanol extract of the

leaves of *Craterispermum schweinfurthi* following phenyl hydrazine induced anemia in male Wistar rats. A total of 40 male wistar rats weighing between 100-250 g were randomly divided into 8 groups of 5 rats each. Phenyl hydrazine was administered in 3 separate doses: 9am on day 0; 9am and 6pm on day 1 to all rat groups except groups 1 and 8. The rat groups were subsequently treated as follows: Group 1 (negative control) received extract vehicle only; Group 2 received 40 mg/kgbw of phenyl hydrazine only; Groups 3-5 received 250, 500 and 750 mg/kgbw respectively of hydromethanol leaves extract of *Craterispermum schweinfurthi*; Group 6 received 0.23ml/kgbw of Bioferon®; both Groups 7 and 8 received 2000mg/kgbw of phytosterol. The extract, Bioferon and phytosterol were administered once daily using oro-gastric cannula for 14 days. On day 15, the rats were placed under chloroform anaesthesia and blood samples collected by direct cardiac puncture into appropriate sample tubes for estimation of erythrocyte count, haemoglobin concentration, haematocrit, platelet count, total white blood cell count and differential counts using automated methods. Amongst Group 2 rats, values of erythrocyte, platelet and leucocyte indices were significantly reduced ($p < 0.05$) following phenyl hydrazine administration compared to Group 1 rats. However, administration of the extract, significantly increased ($p < 0.05$) these parameters in a dose dependent manner amongst Groups 3, 4 and 5 rats. A similar increase was observed amongst Groups 6 and 7 rats administered Bioferon® and phytosterol respectively: suggesting a potential ameliorative effects of the extract comparable to the effects of Bioferon®. Apparently the hydromethanol leaf extract of *Craterispermum schweinfurthi* exhibits potential beneficial dose dependent effects on haematopoiesis in experimental animals. These validates the use of the leaves of the plant in our environment as a tonic. Our findings are preliminary requiring further investigations.

Keywords: *Craterispermum schweinfurthi*, phenyl hydrazine, phytosterol.

AMELIORATIVE EFFECT OF CO-ADMINISTRATION OF CAFFEINE AND MENTHOL ON OXIDATIVE STRESS IN RESERPINE-INDUCED DEPRESSION IN MICE

Abubakar, Z.S.^{1,2}, Alhassan, A.W.¹, Akor-Dewu, M.¹, Sani, D.³, Muhammad, U.A.¹, Yusha'u, Y.¹.

Department of Human Physiology, ¹Ahmadu Bello University, Zaria-Nigeria and ²Yusuf Maitama Sule University, Kano-Nigeria

³Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria-Nigeria.

The projected increase in Depression as the leading global disease burden by 2030, as per WHO projections, emphasizes the limitations of current standard antidepressant therapies for Major Depressive Disorder (MDD). 30% of MDD patients do not respond sufficiently to these treatments, underscoring the need for novel antidepressants or adjuncts to augment their effectiveness. In this study, the combined impact of caffeine and menthol, commonly found in beverages like coffee and tea, on depression using a mouse model was assessed. Both substances individually show

varied effects related to depressive disorders, and understanding their interaction is essential due to their widespread consumption and potential implications for public health. Twenty-four hours post intraperitoneal (IP) delivery of reserpine (6mg/kg), Forced Swim Test (FST) was performed to assess depressive-like behaviour. Following this, menthol and caffeine were orally administered separately and in combination at doses of 25, 50, 100 mg/kg, and fluoxetine 20mg/kg for three consecutive days. The Forced Swim Test was then conducted again after the three-day treatment period. Mice were sacrificed by sudden decapitation and blood was collected for the analysis of Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Reduced Glutathione (GSH). Reserpine(6mg/kg) significantly increased immobility time after 24hrs. Following treatment, there was significant decrease in immobility time in forced swim test after treatment for 3 days. Additionally, a significant decrease in serum levels of MDA and increased levels of SOD and GSH at dose of menthol and caffeine of 50mg/kg and 100mg/kg were observed. The results suggest that co-administration of caffeine and menthol decreased depressive-like behaviours by decreasing oxidative stress, which further supports the potentials of these combination as antidepressants.

Keywords: Reserpine, Depression, Menthol, Caffeine, Oxidative Stress.

ANTI INFLAMMATORY EFFECT OF LYCOPENE ON CARRAGEENAN INDUCED KNEE OSTEOARTHRITIS IN MALE WISTAR RATS

N. Bature*, A. W. Alhassan and A. H. Umar

Department of Human Physiology, Faculty of basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State.

Email: batuensere@gmail.com; Tel: 08023817904

Osteoarthritis is an inflammatory joint disease that is characterized by the presence of inflammatory markers in the blood and synovial fluids resulting in deterioration and loss of articular cartilage with concomitant structural and functional changes in the entire joint. Lycopene is a natural pigment that has been reported to possess anti-inflammatory, antioxidant and anti-proliferative properties. The aim of this study was to evaluate the anti-inflammatory effect of lycopene administration on carrageenan-induced knee osteoarthritis in male Wistar rats. A total of thirty male Wistar rats were used for the study, and were divided into six groups of five rats each as follows: Group I received intra-articular injection of normal saline and 0.1 ml of olive oil which served as vehicle control. Group II received intra-articular injection of carrageenan and 0.1 ml of olive oil and served as negative control. Groups III, IV, V and VI received intra-articular injections of carrageenan (0.02ml of 1% w/v) and 10mg/Kg diclofenac sodium, 5 mg/Kg, 10 mg/Kg and 20 mg/Kg lycopene respectively orally for three weeks. The results showed significantly higher ($p < 0.05$) knee joint diameter in a negative control compared to the vehicle control. Lycopene caused a decrease in knee joint diameter, Neutrophil-Lymphocyte Ratio and Serum Interleukin 1 β , while increasing paw grip latency and Serum Interleukin 10. It can be concluded that Lycopene has anti-inflammatory action that was effective in protecting against knee osteoarthritis.

Keywords: lycopene, carrageenan, knee, osteoarthritis

BIOTIN SUPPLEMENTATION IMPROVED REPRODUCTIVE, METABOLIC AND BIOCHEMICAL PARAMETERS OF POLYCYSTIC OVARIAN SYNDROME IN FEMALE WISTAR RATS.
Onyeso G. I, Emeghara, G, *Reuben, E, Lekara, N.L., Gbarator F.S.

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria.

Email: reuben.edith@ust.edu.ng

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine diseases that has several aspects in terms of pathology such as metabolic, endocrine, reproductive, and psychological. However, the etiology of PCOS remains poorly understood. The study investigated the effects of biotin supplementation on the reproductive, metabolic and biochemical functions in female Wistar rats induced with polycystic ovarian syndrome. Twenty five (25) Female Wistar rats (100- 120g) were randomized into Six groups (n = 5/ group): I (control), II Letrozole-treated: 1mg/kg BW ; III Co-treated with Letrozole 1mg/kg and metformin 2mg/100g BW, IV Co-treated with Letrozole (1mg/kg) and biotin low dose (100mg/kg) BW, V Co-treated with Letrozole (1mg/kg) and biotin high dose (150mg/kg). After Six weeks, samples were collected for biochemical markers (Blood glucose, Lipid profile, Oxidative stress markers), Sex hormone profile (Testosterone, progesterone, oestrogen, follicle stimulating hormone and luteinizing hormone) as well as histology of the ovaries. The results of the study showed that biotin supplementation significantly improved dyslipidemia, and biochemical changes in the PCOS rats as high dose of biotin significantly reduced the blood glucose, and LDL while increasing the HDL. The biotin treated group also showed a statistically significant decrease in the level of luteinizing hormone and increase in the level of progesterone and oestrogen levels when compared to the PCOS group. The observation of primary follicles in the ovaries suggests that the treatment with biotin may be influencing or modulating follicular development. These findings suggest that biotin supplementation may be a potential therapeutic option for improving metabolic and biochemical as well as the reproductive changes associated with PCOS and it has a positive impact on the ovaries in PCOS.

Keywords: Biotin, PCOS, oxidative stress.

ANTIDEPRESSANT AND ANXIOLYTIC-LIKE EFFECTS OF AQUEOUS BARK EXTRACT OF CINNAMON IN A MOUSE MODEL OF CHRONIC STRESS

Yusha'u, Y.^{1*}, Muhammad U.A.¹, Bashir, A.H.¹, Iyima, S.O.¹, Yahaya, I.M.¹, and Mustapha, S.,²

Department of Human Physiology, ¹Ahmadu Bello University Zaria, and ²Federal University Dutse, Nigeria.

Depression and anxiety are prevalent mental health disorders, often coexisting and sharing common symptoms. Antidepressants are one of the most common treatment options available; however, their effects can vary greatly from person to person. Cinnamon is a widely used spice derived from the bark of trees in the *Cinnamomum* genus

that has gained attention for its potential neurological benefits. This study assessed the potential therapeutic effects of aqueous bark extract of cinnamon on depression and anxiety in mice exposed to chronic unpredictable mild stress. Thirty Swiss Albino mice were divided into six groups (n=5). Group I received distilled water (control) 10 mL/Kg; Group II (CUMS) were exposed to chronic unpredictable mild stress without treatment, Groups III, IV, and V received 50, 100, and 200 mg/Kg of cinnamon, respectively; and Group VI received fluoxetine 20mg/kg. Chronic mild stress was applied for 14 days without treatment followed by forced swim test (FST) on day 15, then another two weeks of chronic mild stress exposure with treatment followed before the second FST. Open field test (OFT) and elevated plus maze (EPM) test were used to assess anxiety at the end of the treatment sessions. There was significant increase in immobility time in the groups exposed to chronic mild stress in the first forced swim test when compared to the unexposed group (Group I). However, after treatment a significant ($p < 0.05$) decrease in immobility was observed in groups treated with 100 (Group IV) and 200 mg/Kg (Group V) of cinnamon when compared to the untreated group (Group II) as assessed by the second FST. Cinnamon 50 mg/kg significantly improved rearing in OFT. Although, there was no statistically significant change observed in anxiety-like behaviours in EPM. In conclusion, the results suggest that aqueous bark extract of cinnamon possesses antidepressant-like and anxiolytic-like effects in mice exposed to chronic unpredictable mild stress.

Keywords: Depression; Anxiety; Cinnamon; Forced swim test; Open field test; Elevated plus maze test

THERMOXIDIZED PALM OIL DIET (TPO) INDUCED BIOCHEMICAL DISORDERS IN RATS IS AMELIORATED BY FRESH PALM OIL (FPO) AND VITAMIN E

Imoh Emmanuel Ukoh¹, Idongesit Bassey Umoh², Favour Nyoh Beshel², Enene Esu Ukpai³, Samuel Kelechi Mobisson⁴, Jeremiah Etim Antai²

1. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Federal University Otuoke, Otuoke, Bayelsa State, Nigeria

2. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.

3. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Cross River State University, Ogoja, Cross River State, Nigeria

4. Department of Human Physiology, Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers State, Nigeria

TPO have been reported to possess a deteriorative effect on dietary oils because it changes the physicochemical properties of oil thereby destroying many of its beneficial components. However, human populace consuming TPO is on the increase and there is no recognized remedy to it associated diseases. This study was aimed at evaluating the impacts of FPO and vit E on biochemical indices of TPO fed rat. Sixty albino male rats were grouped into 6 (n=10). Group 2, 3, 4, 5 and 6 were fed TPO, FPO, vit E, TPO + FPO and TPO + vit E respectively. Group 1 acted as the control. 15g

of the oils was added to 85g rat chow to prepare respective diet and vit E administered 200mg/kg/day orally. Group 1-4 were fed for 4 weeks, while TPO fed rats in Group 5 and 6 were further treated with FPO and vit. E for another 4 weeks. At the end of the feeding period, blood samples were collected via cardiac puncture. Serum was used for analysis. Total protein, albumin, globulin, total and conjugated bilirubin levels were spectrophotometrically determined using standard kits. Unconjugated bilirubin estimated by subtracting the conjugated bilirubin from bilirubin. Lipid profile were determined using diagnostic kits. Serum analysis revealed that TPO significantly ($P<0.05$) alter biochemical indices negatively compared to control. But FPO and vit E significantly ($P<0.05$) ameliorated the negative biochemical indices of TPO fed rats. Vitamin E was more potent than FPO in reversing lipid profiles, while FPO significantly ($P<0.05$) reverses total protein and bilirubin levels in TPO fed rats than vit E. FPO and vit E may boost biochemical indices and lessen the associated diseases of TPO fed diet.

Keywords: Thermally oxidized palm oil, vitamin E, fresh palm oil, cholesterol, total protein, cholesterol, conjugated bilirubin.

GESTATIONAL ADMINISTRATION OF AQUEOUS LEAF EXTRACT OF *JATROPHA TANJORENSIS* ALLEVIATES POSTPARTUM-LIKE NEUROBEHAVIOURS IN EXPERIMENTAL RATS

Atim B. Antai¹, Imoh E. Ukoh^{2*}, Sunday A. Bisong¹, Emem A. Edet¹ and Martha I. Johnny¹

1. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.

2. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Federal University Otuoke, Otuoke, Bayelsa State, Nigeria

This study examines the effect of gestational administration of aqueous leaf extract of *Jatropha tanjorensis* (JT) on postpartum-like neurobehavioural outcomes to delineate its possibility as a prophylactic, therapeutic agent in the treatment of postpartum symptoms. Inseminated female rats (120-150g) were grouped into two-control and JT group ($n=10$). Control received 20 ml/kg of distilled water and JT group received 500 mg/kg of JT orally once daily for 21 days in gestation. Non-pregnant rats were excluded from the study. Assessed at postpartum include antidepressant-like (force swim test, FST; tail suspension-test, TST), locomotor (open field test, OFT), anxiolytic-like (elevated plus maze, EPM; light-dark box, LDB), learning and memory (T-maze; novel object recognition task, NORT), social (nest score) and analgesic-like (hot plate test, HPT; tail flick test, TFT) behaviours. JT increased ($P<0.05$) mobility and latency to immobility durations in FST and TST; open arm entry ($P<0.001$) and duration ($P<0.01$) in EPM and light box duration ($P<0.05$) in LDB; locomotion and exploration, but reduced anxiety-like levels in EPM, LDB and OFT. It increased nest score ($P<0.05$); mean retraction time ($P<0.01$) of TFT. JT showed positive score for short- and long-term memory in NORT and improved percentage alternation in T-maze though not significant compared to control. In conclusion, the aqueous extract has a therapeutic effect that reduces postpartum-like depression and anxiety, it improve

locomotor activity. JT can be a preventive and adjuvant therapeutic option for pregnant women.

Keywords: *Jatropha*, postpartum disorder, Emotionality, Pain, Gestation

EVALUATING THE POSSIBLE MECHANISM AND ROLE OF *RAUWOLFIA VOMITORIA* IN IMPROVING MEMORY AND MOTOR COORDINATION IN MICE

*Enene E. Ukpai¹, Agnes I. Odey², Asuquo, J. Okon³, Ikpi D. Ewa⁴, Sunday A. Bisong⁵

¹Department of Physiology, University of Cross River State, Cross River, Nigeria.

²Department of Physiology, Federal University Wukari, Taraba State, Nigeria.

³Department of Physiology, Arthur Jarvis University, Akpabuyo, Cross River, Nigeria.

⁴Department of Physiology, University of Calabar, Calabar, Nigeria.

⁵Department of Physiology, Kampala International University-Western Campus, Uganda.

Author for correspondence: Dr. Enene E. Ukpai, esufrena@gmail.com, Phone: +2348037497594

This study evaluated the possible mechanism and role of *R. vomitoria* in improving memory and motor coordination on three nitropropionic acid (3-NP) induced oxidative stress in mice. Seventy (70) albino mice weighing 19 – 35g divided into 7 groups ($n=10$) were used for the study. The work was carried out in two phases: first is the oxidative stress model which had three groups (control, oxidative stress group without treatment, oxidative stress group treated with *R. vomitoria*). The second phase is the cholinergic model which had 5 groups (control, Atropine treated group 2mg/kg, Atropine + *R. vomitoria* treated group, Neostigmine group 2.5mg/kg, Neostigmine + *R. vomitoria* treated group). Animals had free access to feed and clean drinking water. After administration for 21 days, memory and motor impairment symptoms were evaluated using Morris water maze, Novel object recognition task and Beam walking test. Thereafter, spectrophotometry was used to estimate the degree and expression of biomarkers of oxidative stress. In MWM, the group treated with *R. vomitoria* improved memory and learning better when compared to the control and the 3-NP groups. In the NORT, *R. vomitoria* also showed great improvement in learning and memory. In Beam walking test, *R. vomitoria* still showed tremendous improvement of learning and memory ($p<0.05$) compared to the control and 3-NP groups indicating better motor coordination. For the cholinergic model: in the NORT, Atropine treated group had the least improvement on learning and memory and when compared to other experimental groups there was no significant difference. In the MWM, Atropine treated group had least quadrant retention ($p<0.05$) when compared to the control and other experimental groups. During the beam walking test number of lines crossed and number of foot slips increased in the Atropine treated group but was not significant compared with the experimental groups. The number of reversals was least in the Atropine treated group while the other had increased but when compared to the control group it was not significant. Also, for the oxidative stress model: the

concentration of CAT, SOD, GSHPX were significantly lower ($p < 0.001$) in the 3-NP group compared to the control and lipid per oxidation was significantly higher compared to the control. Higher concentration of CAT, SOD, GSHPX and lower lipid per oxidation ($p < 0.001$) were recorded after treatment with *R. vomitoria*. These findings suggest that treatment with *R. vomitoria* significantly improved learning and memory through the oxidative stress model than through the cholinergic model.

Keywords: *R. vomitoria*, 3-NP, Learning and Memory, Motor Coordination, Oxidative Stress.

THE EFFECT OF EDIBLE CLAY SUSPENSION ON SOME HEMATOLOGICAL INDICES AND HISTOLOGY OF THE LUNGS OF FEMALE WISTAR RATS

^{1,2}*Edeha, D.O., ¹Omigie, M.I. and ²Agoreyo, F.O.

Department of Physiology, School of Basic Medical Sciences, ¹Benson Idahosa University and ²University of Benin, Nigeria Nigeria.

*Correspondence: dedeha@biu.edu.ng; Tel: 08073210782

The purpose of this study was to determine the effect of Edible clay (Eko) on some hematological indices and histology of the lungs of Female Wistar rats. Forty female Wistar rats weighing 150–250 g were sorted into four groups named group 1 to 4 and further subdivided into 2 subgroups tagged week 2 and week 4 with five rats each ($n = 5$). Group 1 was the control and orally received distilled water daily, while Groups 2–4 received various doses of edible clay ranging from 200, 800 and 1600 mg/kg respectively, through the same route for 28 days. Five rats were sacrificed from group 1 to 4 on the second and fourth week. Results showed that there was a significant decrease in Monocyte count in the 3 dose groups, a significant decrease in Granulocyte count for the low and high dose groups and a significant decrease of Platelets count in the medium and high dose groups compared to the control after two weeks of administration of edible clay suspension. After four weeks of treatment, there was a significant decrease in White Blood Cell count and Haematocrit for low dose group, a significant decrease in Mean Corpuscular Volume and Platelets count in medium dose group, and a significant increase in Mean Corpuscular Haemoglobin Concentration in the medium dose group compared to control. Histological evaluation revealed moderate activation of Bronchiolo-alveolar lymphoid aggregates and active interstitial congestion in the low, medium and high dose groups after two weeks of administration of Edible clay. After four weeks, activated cells of the mononuclear phagocyte system, activated lymphoid tissue with vasodilation and dilated bronchiole, and florid lymphoid tissue activation were seen in the low, medium and high dose groups respectively. It can be inferred from this study that the ingestion of Edible clay suspension alters some hematological indices and histology of the lungs of Wistar rats.

Keywords: Edible clay, Hematological Indices, Histology, Lungs.

CAFFEINE CONSUMPTION DISRUPTS SOME REPRODUCTIVE FUNCTIONS OF PERI-PUBERTAL FEMALE WISTAR RATS
*Feranmi Omotayo Odewale, Oluwabukola Esther

Enigbokan, Olamide Abraham Akinsefunmi, Adedayo Oluwadamilola Adesida, Eunice Ogunwole

Department of Physiology, University of Medical Sciences, Ondo City, Ondo, Nigeria

*Corresponding Author: phs192027@unimed.edu.ng

Phone: +2348149764094

Consumption of over-the-counter medicines and caffeinated-beverages is prevalent among adolescents. Caffeine has been associated with infertility. This study investigated the impacts of caffeine consumption on some reproductive functions of peri-pubertal female Wistar rats. Twenty-five peri-pubertal Wistar rats (70 ± 20 g) were randomly divided into five groups ($n=5$). I-control (distilled water), II and III received 120 and 180 mg/Kg/day caffeine orally for 28 days respectively, IV and V received 120 and 180 mg/Kg/day caffeine respectively for 28 days and allowed to recover for same period. Vaginal smears were obtained daily to study estrous cycle which occurs 4-5 days in rats. The animals were sacrificed and the ovaries and uteri were harvested for tissue 8-hydroxyguanosine (8-OHdG), Tumor Necrosis Factor Alpha (TNF- α) and C-reactive protein levels were measured by Enzyme linked immunosorbent assay (ELISA). Data were analyzed using ANOVA and $p < 0.05$ was considered statistically significant. Caffeine caused an increase in the frequency of occurrence of metestrus phase even upon its withdrawal, but reduced the frequency of occurrence of the diestrus phase of the estrous cycle. Caffeine increased uterine 8-OHdG but reduced TNF- α and C-reactive protein in the ovary and uterus. Caffeine consumption adversely alters the reproductive functions of peri-pubertal Wistar rats.

Keywords: Caffeine; Ovary; Uterus; Infertility; Peri-pubertal rats.

AGE AND SEX VARIATIONS OF TEAR FILM ELECTROLYTE SECRETIONS AND TEARS BREAKUP TIME IN HUMANS

*Ohwin E.P¹, Nwangwa E.K¹

¹Delta State University, Faculty of Basic Medical Science, Department of Human Physiology, Abraka, Delta State, Nigeria

*ejirony4life@yahoo.com; +2348037200044

Tear film, a vital fluid that lubricates and provide the ocular surfaces with electrolytes and oxygen. Never the less, abnormalities in tear osmolality leads to tear film instability which precedes dry eye disease, an ocular health condition reportedly found in 5-50% of the global population. This study evaluated the effect of age and sex variations on tear electrolytes and break-up time. Four hundred (400) human subjects, 6 years and above with relatively healthy eyes were grouped into twelve groups based on age and sex. Maximum blink interval (MBI) procedure was carried out monocularly for each subject. Tears was collected with plain glass microcapillary tubes and tear electrolytes (sodium, bicarbonate, potassium, calcium and chloride) and tear glucose levels were determined using spectrophotometer. Age and sex comparison for tear K^+ in females showed a significant increase (5.08 ± 0.65 mMol/L) when compared with males' counterpart (3.54 ± 0.47 mMol/L); and tear Ca^{2+} also significantly increased ($p < 0.05$) in males 'age group 36-45 years (5.81 ± 0.75 mMol/L) when compared with

corresponding females ($3.12 \pm 0.62 \text{ mEq/L}$) subjects. Furthermore, tear Na^+ level significantly increased ($p < 0.05$) in females between 45–59 years ($166.38 \pm 16.67 \text{ mEq/L}$) when compared with corresponding males ($127.71 \pm 9.64 \text{ mEq/L}$) while glucose level was significantly ($p < 0.05$) decreased in females between 45–59 years ($14.71 \pm 2.77 \text{ mg/dl}$) when compared with corresponding males ($28.89 \pm 5.14 \text{ mg/dl}$) subjects. Finally, MBI increased significantly for male age group 13–19 years and 20–35 years but also decreased significantly ($p < 0.05$) for group 36–45 years in female. Comparison of average MBI values for sex at different age groups was only significant ($p < 0.05$) for 13–19 years. MBI had a weak positive correlation with K^+ , Cl^- , Ca^{2+} , Na^+ , and HCO_3^- concentration but not ($p < 0.05$) significant. However, had a significant ($p < 0.05$) weak negative relationship with tear glucose. Conclusively, the study found that tears stability is age dependent but not gender related.

KEYWORD: Tears film, Electrolytes, Tears Breakup Time, Glucose, Age, Gender, Potassium, Sodium, Chloride, Bicarbonate.

ANTI-HEPATOTOXIC ACTIVITIES OF METHANOL FRUIT PULP EXTRACT OF *Azanza garckeana* IN EXPERIMENTALLY-INDUCED TYPE II DIABETIC RATS

*Farrau, U.¹, Tanko, Y.¹, Augustine, B.D.¹, Jaafar, A.², Sulaiman, M.G.¹, Kasim, K.U.¹, Abubakar, F.S.¹, Moussa, M.K.M.M.¹ and Wahab, A.O.²

¹Departments of Human Physiology and ²Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria

* usmanfarrau@gmail.com, +2348032892573

Diabetic hepatotoxicity is a microvascular complication of diabetes mellitus characterised by a damage to the liver majorly as a result of hyperglycemia. This study investigated the effects of methanol fruit pulp extract of *Azanza garckeana* (MFEAG) on liver tissue liver enzymes and oxidative stress biomarkers in experimentally-induced Type II diabetic male Wistar rats. Thirty rats (weighing 70–100 g) were grouped into six ($n = 5$); normal control, diabetic control, 300, 600 and 1,200 mg/kg MFEAG- and metformin-treated groups respectively. Type II diabetes was induced by feeding the rats with high fat diet and water *ad libitum* for five weeks followed by administration of single low dose of streptozotocin (STZ, 40 mg/kg, i.p.) while normal control rats were fed with normal diet and STZ vehicle. Treatment was done orally for three weeks, with the diabetic control group receiving the extract's vehicle. Treatment with MFEAG significantly reversed an increase in levels of aspartate aminotransferase (AST) and malondialdehyde (MDA), while significantly reversing a decrease in levels of superoxide dismutase (SOD) and glutathione reductase (GSH) in the rats' liver homogenates. No effects were observed in the levels of alanine aminotransferase (ALT) and catalase (CAT). Histology of liver tissues of a diabetic control rat shows moderate vacuolation and necrosis, while those of the treated rats indicate normal features comparable to that of a normal control. These findings suggest that the methanol fruit pulp extract of *Azanza garckeana* possesses some abilities to reverse the hepatotoxic effects of experimentally induced type II diabetes mellitus in rats.

Key words: Diabetes, hepatotoxicity, *Azanza garckeana*, liver enzymes, oxidative stress, rat

EVALUATION OF THE EFFECT OF ETHANOLIC LEAF EXTRACT OF *JUSTICIA SECUNDA* ON OXIDATIVE STRESS AND RENAL FUNCTION IN ALLOXAN-INDUCED DIABETES.

Chidinma Winifred Chukwukaeme¹, Mary Igbokwe², Jeffrey Ndubueze, Vincent Umeanozie¹, Ifeoma Okeke¹, Ifeoma Okwuonu¹, Anulika Umezulike¹, Kester Nwaefulu

Department of Physiology, ¹Nnamdi Azikiwe University Awka and David Umahi Federal University of Health Sciences, Uburu

Email: chukwukaemechidinma@gmail.com

The statistics of Nigerians living with Diabetes Mellitus (DM) are disturbing as the current prevalence among adults aged 20–69 years is reported to be 1.7 million. Oxidative stress has been implicated in the pathophysiology of DM. Persistent hyperglycemia causes increased production of free radicals in tissues and can cause renal damage/injury over time. Medicinal herbs like *Justicia secunda* are being explored as alternatives to orthodox medicine as they are less toxic and eco-friendly. Twenty-five rats (150–200g) were divided into 5 groups. Diabetes was induced in Groups B–E with 150mg of Alloxan Monohydrate (IP). Group A (Normal) and Group B (Untreated Diabetes) received only feed and water, Group C received 150mg/kg of metformin while Group D and E received 200mg/kg and 400mg/kg ethanolic extract of *Justicia secunda* respectively for 14 days. *Justicia secunda* significantly reduced MDA levels and significantly increased SOD and Catalase levels. *Justicia secunda* significantly decreased urea levels while creatinine levels were unaltered. *Justicia secunda* showed antioxidant potential and was not toxic to the kidney, suggesting that it may be safe for consumption by diabetic patients.

Keywords: Diabetes mellitus, Oxidative stress, *Justicia secunda*, Antioxidants.

MODULATION OF CELL ADHESION MOLECULES AND LEPTIN BY AQUEOUS EXTRACT OF *Terminalia catappa* (ALMOND TREE) LEAVES IN ALLOXAN – INDUCED DIABETIC MODEL.

Ezekiel E. Ben^{1,4}, Itemobong S. Ekaidem², Sampson A. Oyebadeyo³

Departments of ¹Physiology and ²Chemical Pathology, University of Uyo, Uyo, Akwa Ibom State.

³Department of Biomedical Laboratory Sciences, Faculty of Fundamental Applied Sciences, Institut d'Enseignement Supérieur de Ruhengeri, Musenze District Northern Region, Rwanda

⁴Department of Physiology, Prince Abubaka Audu University, Anyigba, Kogi State

Cell adhesion molecules (CAMs) are subset of cell surface proteins involved in the binding of cells with other cells. The influence of *Terminalia catappa* leave extract on soluble cell adhesion molecules and leptin in diabetic rat model was investigated. Male Wistar rats weighing 150 – 200g were randomly distributed into five groups of six rats each. Group

1 was control administered distilled water orally at 5ml/kg bw. Group 2 received *Terminalia catappa* extract at 130mg/Kg bw orally. Diabetes groups 3, 4 and 5 were administered orally with distilled water, 5ml/Kg bw; *Terminalia catappa* 130mg/Kg bw and aspirin 30mg/kg bw respectively. Diabetic rats demonstrated significantly ($p<0.05$) higher levels of ICAM ($326\pm1.14\text{mg/ml}$), VCAM ($451.4\pm0.93\text{ mg/ml}$) and P-selectin ($450.6\pm0.27\text{mg/ml}$) compared with control values $91.2\pm0.58\text{mg/ml}$, $234.8\pm1.2\text{mg/ml}$ and $33\pm0.6\text{mg/ml}$ respectively. Leptin also increased significantly ($p<0.05$) from $9.6\pm0.98\text{mg/ml}$ in control to $31.4\pm0.51\text{mg/ml}$ in diabetic group and reduced to $17.5\pm0.96\text{mg/ml}$ in diabetic treated group. In diabetic rats treated with extract, the cell adhesion molecules were significantly ($p<0.05$) lower than values of untreated diabetic but higher than those of control. Aspirin also lowered the concentration of cell adhesions molecules. *T. catappa* reduction of adhesion molecules and leptin can be harnessed for the management of cardiovascular complications in diabetes mellitus and other disease conditions.

Keywords: soluble adhesion molecules, ICAM, VCAM, diabetes mellitus, cardiovascular complications

AQUEOUS STEM EXTRACT OF COSTUS AFER (BUSH CANE) AMELIORATES ETHANOL-INDUCED MEMORY DEFICITS AND ENHANCES BRAIN ACETYLCHOLINESTERASE (ACHE) ACTIVITY IN MICE.

*Agnes Igimi Odey¹, Iliya Ezekiel¹, Ejike Daniel Eze², Enene Esu Ukpai³, Obioha Mary⁴ and Sunday Agba Bisong⁵

¹Department of Physiology, Faculty of Basic Medical Sciences, Federal University Wukari, Taraba State, Nigeria.

² Department of Physiology, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda.

³Department of Physiology, Faculty of Basic Medical Sciences, University of Cross River State, Nigeria.

⁴ Department of Biological Sciences, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, Nigeria.

⁵Department of Physiology, Kampala International University-Western Campus, Uganda

*Email: igimiodey@gmail.com, Phone: +07060640063

Numerous studies have revealed the adverse effects of alcohol on memory. Despite being recognized to cause both acute and long-term cognitive deficits, alcohol is one of the most commonly abused psychoactive drugs in our society. Hence, the current study explores the neural processes behind the protective action of *Costus afer* (*C. afer*) in ethanol-induced memory losses in mice. The animals were weight-matched into 5 groups ($n = 9$) as follows: Control, ethanol (5 g/kg b w), ethanol plus *C. AFER* 100 ml/kg b w, ethanol plus *C. AFER* 200 ml/kg b w, and ethanol plus *C. AFER* 500 ml/kg b w groups. The Morris Water Maze (MWM) and Novel Object Recognition Test (NORT) were used to evaluate cognitive impairment. Furthermore, levels of malondialdehyde (MDA) as a lipid peroxidation maker of oxidative stress, antioxidant capacity, and alterations in acetylcholinesterase (AChE) activity were assessed in mice

exposed to ethanol. Treatment with *C. afer* significantly improved antioxidant defense system (CAT and GSH) and balanced the elevated MDA levels in the ethanol-induced mice. At the same time, this study showed reduced AChE activity in the mice brain, thereby enhancing cholinergic neurotransmission. These findings coincided with an improvement in the MWM and NORT's performance in both spatial and non-spatial memory. The findings of this investigation demonstrate that *C. afer* has a positive therapeutic effect in acute ethanol consumption by reducing oxidative stress and cholinergic inhibition.

Keywords: Ethanol; *C. afer*; Oxidative stress; Acetylcholinesterase; Spatial memory; Non-spatial memory

PREGNANCY OUTCOMES OF *Rauvolfia vomitoria* STEM EXTRACT TREATED WISTAR RATS.

¹Nonju I.I, ¹Obiandu .C, ¹Gbaranor K.I, ¹Wami-Amadi.C, ¹Orijio E.I, ²Nonju T.I , ¹Otto. J and ³Obomanu.T.R

¹Department of Human Physiology ,College of Basic Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

²Department of Pharmacology, College of Basic Clinical Sciences , Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

Department of Medical Biochemistry, College of Basic medical Sciences, University of Port Harcourt, Rivers State ,Nigeria

Email: tamunobarabiye.nonju@ust.edu.ng

Phytomedicine is a globally accepted means of providing alternative treatment for a varied number of disease conditions. *Rauvolfia vomitoria*, a medicinal plant locally called "poison devil's pepper", is used in traditional medicine for various treatment of diseases such as hypertension, epilepsy, cancer, psychosis, diabetes etc. The ethanolic extraction of *Rauvolfia vomitoria* was carefully carried out and the extract administered to twenty rats, which were divided into four (4) groups. Group A (control) received 0.5ml/kg body weight of distilled water. Groups B, C and D received 200mg/kg, 400mg/kg and 800mg/kg body weight of *Rauvolfia vomitoria* stem extract respectively by oral gavage for 19 days. Hormonal assay for progesterone, estrogen, luteinizing hormone, follicle stimulating hormone and prolactin were done using enzyme immunoassay. Findings from this study revealed that *Rauvolfia vomitoria* stem extract caused a significant decrease ($p<0.05$) in relative change in body weight, the weight of foetuses was significantly decreased ($p<0.05$) but the weight of the ovary and uterus of the rats had no noticeable change. Serum level of follicle stimulating hormone (FSH) had a significant decrease where as a significant increase in luteinizing hormone (LH) occurred at 200mg/kg and a decrease at 400 and 800mg/kg. Serum prolactin level had no significant difference however it decreased in all groups compare to control. There was an initial significant increase ($p<0.05$) in the serum levels of estrogen in 200mg and significant decrease in 400mg/kg groups, relative to the control. There was no observable death in foetuses delivered but resorption was significant at 800mg/kg. Histopathology findings showed more dose dependent degeneration, atretic cells in the ovary and glandular distortion of the uterus at 800mg/kg. The levels of glutathione Peroxidase (GPx) and catalase

(CAT) decreased significantly while the extract had no significant effect on superoxide dismutase (SOD) and malondialdehyde (MDA). The findings suggest that *Rauvolfia vomitoria* stem may possess a dose dependent abortifacient effect in pregnant rats.

Keywords: *Rauvolfia vomitoria*, Pregnancy, Abortifacient, sex hormones, Antioxidants, gonadotrophins

MODULATORY ROLE OF QUERCETIN ON ANXIETY, DEPRESSION AND OXIDATIVE CHANGES IN PARAQUAT-INDUCED PARKINSONISM IN MALE WISTAR RATS

Umar, H.A., Adebola, A.P., Sunday, N., Rufa'I, H.B., Abdulkarim, H.A., Yakubu, H.M., Muhd, N.G., and Bouey, M.I.

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria.

***Email:** hajara.ali.umar@gmail.com; +234(0)8035073833

Parkinsonism is a complex neurodegenerative disorder characterized by motor and non-motor symptoms with signs of prominent bradykinesia and variable extrapyramidal signs and symptoms, accompanied by the degeneration of nigrostriatal dopaminergic system, with neuronal loss of the substantia nigra. Toxins are implicated in the etiology of the disorder. Anxiety, depression and oxidative stress are

common comorbidities, significantly affecting the quality of life in parkinsonian patients. Quercetin a naturally occurring flavonoid found in common fruits and vegetables such as spring onions, tea and red wine, with antioxidant properties, has shown promise as a potential modulator of these symptoms. This study aimed to investigate the modulatory effect of quercetin on anxiety, depression and oxidative changes in paraquat-induced parkinsonism in male Wistar rat model. Twenty (20) male Wistar rats were divided into four (4) groups of five (5) rats each. Group 1 rats received distilled water only, group 2 received tween 80, group 3 received paraquat (15mg/kg) and group four received paraquat (15mg/kg) followed by quercetin (40mg/kg) after 30minutes. Anxiety levels were assessed using established behavioral tests (open field and elevated plus maze tests); depressive like symptoms were also assessed using the tail suspension test at the end of weeks 1 and 2 of drug administration, while oxidative stress markers (MDA, CAT and SOD) were analyzed after termination of the study. The results obtained showed a significant reduction in both anxiety-like and depressive like behaviors and an increase in antioxidant activity in rats treated with quercetin, suggesting a modulatory role of quercetin in alleviating mood alteration and oxidative changes associated with parkinsonism.

Keywords: Parkinsonism, quercetin, paraquat, depression, anxiety, oxidative stress.

Review Article

The Translational Physiologist: Strengthening Research to Enhance Quality of Life

***Jaja S.I.**

Department of Physiology, Faculty of Basic Medical Science College of Medicine University of Lagos.

Summary: Translational research and the Translational Physiologist are new concepts but important for optimizing quality of life of citizens. This paper reviews the origins of the concepts, phases, partnerships and critical factors in translational research. Some barriers to proper functioning of the Translational Physiologist in Nigeria and the role the Translational Physiologist in repurposing are discussed. The paper highlights collaboration between the Translational Physiologist and other specialists to attain optimal health and enhanced quality of life of the citizens.

Keywords: Bench to bedside; Reverse translation; Translational research; Translational Physiologist.

*Current address: Department of Physiology, Faculty of Basic Medical Sciences, PAMO University of Medical Sciences, Port-Harcourt, Rivers State, Nigeria. Email: sjaja@unilag.edu.ng, Tel: ++234-8036729192

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INTRODUCTION

Translational physiology is a very recent concept that was suggested by John Hall in 2002 (Hall, 2002) and derives from translational research but with special emphasis on the physiological sciences. It widens the scope of physiology from the traditional areas of integrative and clinical physiology and extends the investigative scope of basic research to community health. It is therefore a model for physiological investigation from molecular and cellular physiology to population physiology and public health (Seals, 2013). Thus, while integrative physiology aims to connect observations from subcellular events to the whole organism, and clinical physiology focuses on specific groups of patients and healthy controls, translational physiology extends the study of physiological functions from the molecular level to populations of humans with application to public health. Integrative and clinical physiology, however, remain foundational parts of translational physiology (Seals, 2013). The aim of translational physiology is to enhance the societal impact of physiology by promoting and strengthening its role in biomedical research (Seals, 2013).

Currently in Nigeria, physiology is taught in the traditional integrative physiology mode with some addition of clinical physiology. Most researchers conduct their business in silos and results of such studies often end up on the shelf without benefit to the general populace. Translational physiology seeks to extend the traditional integrative physiology into the populace and therefore into public health. In other words, translational physiology extends from molecular and cellular levels through organ/system physiology, the organism into population

studies and public health. Translational physiology, therefore, seeks to expand the frontiers of physiology and reorientate the way research in physiology is designed and executed. To establish and promote translational physiology, physiologists must be properly trained and oriented. Such training must inculcate the philosophy and methodologies of translational research (as described below) and public health. Translation physiologists must also inherit and grapple with the challenges and problems associated with translational research in a developing country. More effort should be made at integrating the traditional integrative physiology to population physiology and public health. Translational physiologists will need to embrace and employ the tools and techniques of population studies and public health research like epidemiology, behavioural science and public policy including those that are needed for synthesizing, disseminating and implementing observations made at the clinical research level to the community. The tools include systematic reviews, meta-analyses and medical guidelines development. Since translational physiology derives from translational research, what then is translational research?

Origin and Basic Concepts of Translational Research:

Translational research started in the early 1990s (Curry, 2008). Originally, it was associated with biology-based attempts at finding new drugs for the treatment of cancer (Sugarman and McKenna, 2003). Later it got associated with other disciplines of medicine like cardiology, neurology, psychiatry and pathology (Cripe *et al*, 2005). Several terminologies have been used to describe translational research. These include "Bridging the chasm", "walking the bridge," "lost in translation," and "Bridging the gap,"

Presently, it is referred to as “from bench to bedside” (Bench 2 Bedside) (Farrel *et al*, 2019). Translational research encourages and promotes multidisciplinary collaboration between Basic Medical Scientists and Clinical Researchers and thus is aimed at converting results from basic research into activities that directly benefit society. It brings together biomedical scientists, physicians, bioengineers, epidemiologists, patent experts, and several other specialists to achieve the purpose of advancing health care. Translational research identifies and supports the adoption of best medical and health practices for the good of the patient or general public. In addition, translational research encourages academic participation in drug research and development. On the other hand, translational science focusses on unraveling the operational principles underlying the translational process (Gillard *et al*, 2019).

Relationships in translational research

Basic Medical Science research is the foundation of medical discovery. Through it, key information about the fundamental molecular, biological and chemical processes of life are obtained. Clinical trials involve testing new methods of diagnosis, prevention, and treatment of illness using human subjects. It aims to determine whether the new procedure or drug is safe and effective. Through clinical trials, new medications, medication combinations, new uses for existing medications and medical devices are evaluated. Translational research serves as the bridge between basic medical research and clinical trials. It transforms scientific ideas and discoveries into new treatments and approaches to medical care with the aim of improving the health of the population. Examples of translational research include drug development, studies of disease mechanisms, including proteomics, genomics, genetics, metabolomics, and animal models.

Observational studies, such as case reports do not qualify as translational research. Quality of life studies or comparative treatment studies involving patient cohorts in which there is no adequate control group have limited translational value due to limitations in study design. Translational research is broader than the term “applied research”. Applied research is any research that may possibly be useful for enhancing health or well-being. It does not necessarily have to have any effort connected with it to take the research to a practical level. An example of applied research is demonstrated in the study of Jaja *et al*, (2002). In Nigeria, the traditional way of carrying children for extended periods and long distances is on the back. In Europe and America, women prefer to carry the child with the aid of a suspender on the chest when they are not pushing the baby around in a pram. The researchers investigated the energy conserving implications of carrying weight on the back or on the chest. Ten (10) healthy females between the ages of 19.0 years and 24.0 years were recruited for the study. The control experiment consisted of the measurement of arterial blood pressure (mm hg), pulse rate (beats/min) and oxygen consumption (l/min) and the calculation of energy expenditure in each subject after walking a distance of 76.5 meters in two (2) minutes (without carrying any load). Experiments were then repeated with a load of 6.4 kg (average weight of a 6-month-old baby) carried on the chest with a suspender or tied to the back respectively after one week interval. Results showed that pulse rate, oxygen

consumption and energy expenditure were significantly higher when weights were borne on the chest than on the back and showed that carrying babies on the chest was more energy demanding and thus less efficient than carrying the baby on the back. The study thus validated the age-old African tradition of carrying babies on the back.

partnerships in translational research

Translational research involves partnerships. Figure 1 shows the partnerships in translational research. The central operators are the translational researchers that include the translational physiologists, medical doctors and any other interested and qualified researcher. The physiologist serves as the expert on the bench and is responsible for all results obtained from the laboratory. The medical doctor in the team assists in the recruitment of patients from the outpatient clinic or ward of an approved hospital for the study. The translational researchers will also work with other health practitioners that are not members of the team, especially to ensure orderly recruitment of patients or subjects. These practitioners will also ensure that the patients or subjects are properly catered for. The principal investigator (pi) emerges from this group and is responsible for general management of the project throughout its duration.

Another arm of the relationship is the patients or subjects or participants. This group includes the control and the test subjects. Participants must understand the aims of the study and must be accorded with the greatest respect. All participants of a study must sign a consent form and ethical approval must also be obtained from the relevant institutional review board or the experimentation ethics committee of the institution before the study begins. Where necessary, each participant will have to fill out a well-structured questionnaire that will obtain personal information concerning the participant. Information obtained from such questionnaire are held in strict confidence.

Funding is very important for successful research. Thus, translational researchers must seek out and work with donor agencies for adequate funding. Donor or grant awarding agencies, which may be local or foreign, may include the pharmaceutical industry, Tertiary Education Trust Fund (TETFUND), the parent university, philanthropic organizations and individuals. Pharmaceutical companies could be important allies for the provision of research funds especially for clinical trials. They could also be important in manufacture and marketing of the final product.

The research team will also deal with regulatory organizations like the National Agency for Food and Drug Administration and Control (NAFDAC). This agency is responsible for protocol review and authorization of clinical trials before they are conducted in Nigeria. It is also responsible for carrying out inspection of trial sites to monitor the conduct of authorized studies to ensure that the well-being and safety of the participants are protected and that data obtained are credible. The National Health Research Ethics Committee (NHREC) is responsible for accreditation of the Independent Ethics Committee (IEC) and/or Institutional Review Board (IRB) of different institutions that give ethical opinion on study protocols. Researchers will obtain approval of institutional review boards and ethical committees in their institutions before studies can commence.

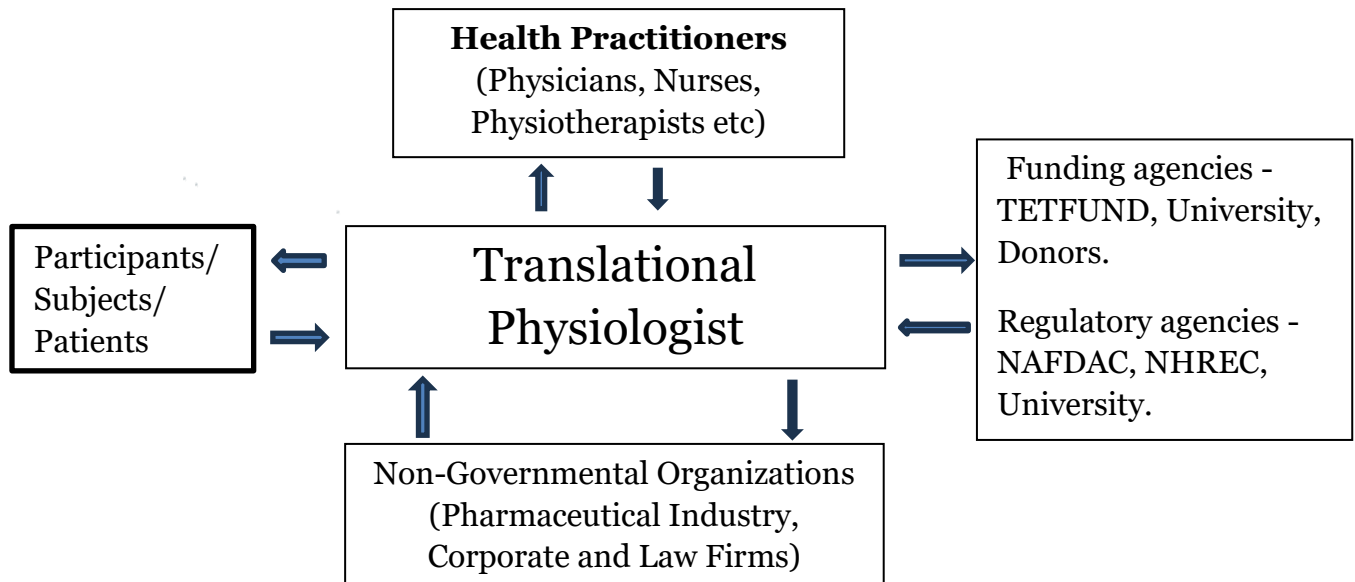


Figure 1.
Partnerships in translational research.

Critical factors in translational research. The following factors are critical in translational research.

Excellence in science. Any person that intends to be part of a translational research team must have a thorough knowledge of the subject. The researcher must also possess adequate and/ or relevant laboratory skills or techniques to deploy for the study. The individual must also have the ability to interpret data. The translational physiologist must possess adequate knowledge and training in experimental design and data analysis. The scientist should be able to conduct research that ensures transparency and reproducibility of his work.

Ability to traverse domains. The translational physiologist should be able to collaborate with others and break down disciplinary boundaries to collectively advance the development of a medical intervention. being a translational scientist requires venturing beyond one's own research discipline or professional domain to gain an understanding of the fundamental knowledge, methodologies, and priorities of all other disciplines and domains involved in a translational research project. an effective translational scientist should be an interpreter of the multiple languages of biomedical, clinical, and public health research for those translational team members caught up in their silos. in other words, a translational researcher or scientist should have expertise in one or more translational domains to be able to contribute novel solutions to complex problems.

Ability to manage interpersonal relationships. Since translation research involves working as a team, it is imperative that all members of the team function in harmony. Any person involved in translation research must be able to manage interpersonal relationships. Courtesy and friendliness towards each other are essential ingredients of teamwork. Even where disagreements arise, effort must be quickly made to amicably settle such. Disharmonious interpersonal relationships and rivalry will impede the success of the study.

Skilled communicator: The translational physiologist/researcher must have the ability to communicate clearly to all stakeholders across diverse social, cultural, economic and scientific backgrounds. This includes colleagues in other fields, patients, grants donors and reviewers. Skilled communication involves that the researcher is able to write and speak in a manner that there is no ambiguity about the researcher's thoughts.

Project management. Although there will be a team leader or principal investigator, each member of the team should have a clear understanding of the objectives and his/her place in contributing to the success of the general plan. The team leader should be able to assign roles and responsibilities to team members and manage progress throughout the project's duration. The timelines, budget and scope of the project must be adhered to and tasks completed within the guidelines.

In complex projects, it may be essential to seek assistance of project managers who would ensure order. This is essential when a team of equal people from different disciplines relate to each other in a horizontal hierarchy. The functions of the project managers would include the preparation of a target product profile, providing work breakdown structures, managing external liaisons, and providing structure to projects, especially in regard to dependencies and bottlenecks, identification of milestones, and other stage markers. other functions would be the provision of templates to ensure timely and adequate recording of data and to organise team meetings. These types of activities may not be suited to the average university researcher but essential in translational research.

Phases of translational research: Translational research is made up of phases labelled phase 1 to phase 3 or t1 – t3. Phase 1 or t1 refers to the development of concepts and discoveries from basic research through early phase clinical trials (bench to bedside) (Homer-vanniasinkam and Tsui, 2012). It requires basic science skills, some clinical skills and appropriate laboratory infrastructure. Phase 2 extends the results or observations obtained in t1 by establishing the

efficacy in humans. In phase 2 or t2, observations from clinical research are extended into medical and community practice (bedside to community). Phase 2 research needs skills in behavioral science and epidemiology research. Phase 3 or t3 of translational research is the implementation and dissemination into the community of phase 2 research results (clinical practice to community or population health) and involves the ability to influence public policy (Woolf, 2008; Homer-vanniasinkam and Tsui, 2012). Application of research results to the community may stimulate further information that can lead to further research, this time from the bedside to the bench referred to as “reverse translation” (Marincola, 2003; Homer-vanniasinkam and Tsui, 2012; Liebman and Marincola, 2012; Van der laan and Boenink, 2012).

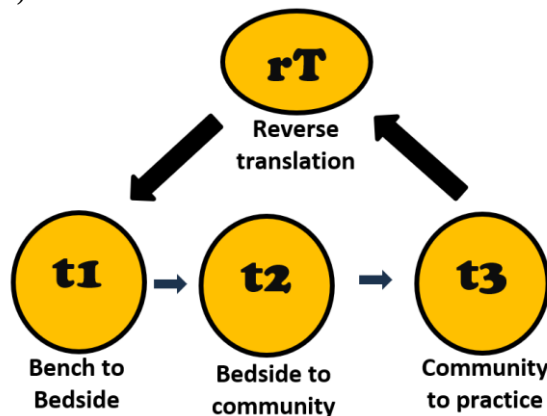


Figure 2
Phases in translational research

Barriers to translational research in Nigeria: There are several barriers, impediments or hurdles to the successful implementation of translational research in Nigeria.

Lack of funds: First and very important is the lack of funds for research. Researchers should be able to attract grants for funding research from public and private sources. the only meaningful source of government funding for research in Nigeria is through the Tertiary Education Trust Fund (TETFUND), funded by the federal government of Nigeria. There is little or no support for research from different state or local governments. corporate organizations and private individuals are also not providing financial support for research especially with the instability in the Nigerian economy. Researchers are then left with supporting research with personal funds. This cannot provide meaningful financial support for translational research. Research support from international donors is very scanty and when available may not go far enough to support long-term studies in translational research.

Collaborations with international partners are of great benefit to local research capacity building. These partnerships are expected to provide funding, technical assistance, and mentorship, further strengthening local research capabilities (Olatunji *et al*, 2023). The transfer of knowledge, expertise, and technology from international partners to local researchers contributes to the overall growth and advancement of research in the country. The ultimate goal of these collective efforts is to empower local researchers and institutions to conduct impactful research that addresses the specific health challenges of local

populations. by investing in research infrastructure and capacity building, Nigerian research institutions can generate locally relevant evidence, inform health policies, and improve health-care delivery. These efforts are expected to result in better health outcomes for our local populations. International donors and collaborators are few and when available dictate or cater to their own research needs which may not necessarily support translational research direction, efforts and needs in Nigeria.

Research culture and interest: Lack of funds demotivates interest in research (Salami and Kanmode, 2021). Lack of or reduced interest in research further leads to reduced research culture in the universities. Although the functions of the university lecturer are 3-fold, - research, teaching and community service, - the university lecturer focuses more on the latter two than on research due to demotivation.

Lack of collaboration among researchers: Translational research requires collaboration among researchers (Olatunji *et al*, 2023). Working alone or in silos therefore becomes an anathema. Translational research is multidisciplinary and depends on a network of researchers working collaboratively. Basic science largely seeks to acquire knowledge while translational research seeks to improve health. The successful translational physiologist needs to be comfortable in both of these cultures, be fluent in many fields, and thrive on collaboration. Nigerian researchers must therefore embrace collaboration with other researchers in order to improve health care in the country (Salami and Kanmode, 2021).

Prolonged work stoppages: Work stoppages in Nigerian universities contribute to demotivating research. Work stoppages arise from disputes between various university trade unions and the federal or state governments or between the university-based unions and the university management. The length of work stoppages had varied from a few days to a year. in the period of the stoppage, little or no research is done. in some cases, salaries of staff had been withheld and researchers abandoned to their own means. This further contributes to dampening the morale of the researcher. Sometimes, expensive reagents are lost to work stoppages, and this affects research activity and output.

Economic uncertainty. Economic uncertainty in Nigeria has a great impact on research in Nigeria (Salami and Kanmode, 2021). Instability in the foreign exchange regime is detrimental to trade. Over time and sometimes in a frame of a few days the local currency, the naira, losses value in favor of foreign currencies which impacts negatively on the cost of reagents and equipment vital to research. Without a stable foreign exchange regime, it becomes difficult to plan and execute any meaningful research because it is difficult or impossible to buy or import any meaningful research equipment or reagent that is priced in foreign currencies. a stable foreign exchange climate is therefore necessary for meaningful research in Nigeria.

Brain drain: The issue of brain-drain due to frustration arising from one or a combination of factors enumerated above limits capacity for translational research. brain-drain constitutes a loss of valuable local expertise (Olatunji *et al*,

2023). the exodus of talent from research institutions in Nigeria to more stable areas of the world rob the local institutions the expertise required to innovate and further develop or deepen translational research in our local environment. the weakened research capacity and capability of the institutions effectively impedes the ability of research institutions to contribute meaningfully to solving health challenges in Nigeria. translational research will not have its maximum beneficial impact in Nigeria if skilled researchers continue to migrate to areas of better opportunities outside the country.

Lack of interest by policy makers. Another barrier that had been identified as hindering translational research is the reluctance of policy makers to accept and adopt local research findings into policy. policymakers play a critical role in the translation of research findings into policy but may not be appropriately equipped for the task. The limited capacity of policymakers to use evidence had resulted in delayed adoption of research findings for policy formulation. Local policy makers rely on international organizations for direction and therefore are reluctant to embrace locally produced research findings and turning them into policy (Damba *et al* 2022).

Translational research and repurposing: Nigeria is very rich with a variety of plants and herbs which traditional healers had employed in folklore medicine. These concoctions or decoctions are obtained from plant sources. While some of the mechanisms of action of these traditional medications had been worked out, a lot of the mechanisms of action of others are still not well understood. translational physiology research affords the opportunity of validating and adopting some of the medicines from plant sources into modern treatments of diseases like diabetes mellitus, asthma, cancer, sickle cell disease, alzheimer's and parkinson's disease. Translational physiologists are well suited to identify, characterize and quantify the chemical composition of identified leaves, roots, stems that are needed to treat the various ailments ravaging mankind (Salisu *et al*, 2019; 2022).

Translational physiology research can play a role in drug repurposing or drug repositioning. drug repurposing refers to research efforts at finding new therapeutic uses for currently approved drugs or agents that had previously been withdrawn, or relatively out-dated drugs (Akindele *et al*, 2022). Successes had been recorded in this regard and a few examples include sildenafil which was used in the treatment of angina but is currently used to treat erectile dysfunction. Other examples include the repurposing of aspirin as an analgesic to the treatment of colorectal cancer and topiramate originally used to treat epilepsy and now applied in treatment of obesity (akindele *et al*, 2022).

Antioxidants such as l-arginine have been studied to determine their beneficial effect in the management of sickle cell disease (Jaja *et al*, 2016; Saka *et al*, 2023). Sickle cell disease (SCD), a group of multisystem autosomally recessive inherited hemoglobin disorders, is caused by a point mutation in the gene encoding β chains of hemoglobin. this disease is highly prevalent in Africa, and Nigeria contributes the greatest number of sufferers (Diallo and Tchernia, 2002; Piel *et al*, 2013). Sadeghi *et al*, (2023) carried out a systematic review and meta-analysis assessing

the effect of the use of l-arginine in patients with SCD. The study confirmed that the use of l-arginine could be beneficial in the management of SCD. However, it was recommended that for a firm conclusion and rationale for arginine therapy for these patients, more studies with larger population sample sizes, longer follow-up durations, and well- developed designs were needed. Successful clinical trials, acceptance and adoption of l-arginine for the management of sickle cell disease will be another example of repurposing or repositioning.

CONCLUSION

Translational research and translational physiology are new areas of endeavor and specialization. In order to serve the community adequately, Nigerian universities must recognize the place of translational research in modern day scheme of things. investing in translational research will bring a lot of financial rewards to the university and her staff through patents. On their own part, translational physiologists will contribute to the attainment and preservation of optimal societal function, health and quality of life and solidify the critical role of the subject in the biomedical research enterprise. To achieve these objectives, the translational physiologist must collaborate with other researchers from diverse fields. Collaboration is the driver of translational research and the essence of translational physiology.

REFERENCES

- Akindele A. J., Fatokun A. A., Uzochukwu I. C., Steenkamp V., Agyare C., Familoni O. And James M. O. (2022). Editorial: Translational Research and Drug Repurposing For Non-Communicable Diseases (Ncds). Front. Pharmacol., Sec. Translational Pharmacology. 13; 202.
- Cripe, T.P., Thomson B., Boat T.F. And Williams D.A. (2005). Promoting Translational Research in Academic Health Centers: Navigating The "Roadmap." Acad. Med. 80:1012-1018.
- Curry Sh (2008). Translational Science: Past, Present, And Future. Biotechniques For Preclinical Development. 44(2); I-Viii.
- Damba F.U., Mtshali N.G. And Chimbari M.J. (2022). Barriers And Facilitators Of Translating Health Research Findings Into Policy In Sub-Saharan Africa: A Scoping Review. Humanities Soc. Sci. Coms. 9:65.
- Farrell A.T., Panepinto P., Desai A.A., Kassim A.A., Lebensburger J., Walters M.C. *Et Al*. (2019). End Points For Sickle Cell Disease Clinical Trials: Renal And Cardiopulmonary, Cure, And Low-Resource Settings. Blood Advances. 3(23): 4002-4020.
- Gilliland C. T., White J., Gee B., Kreeftmeijer-Vegter R., Bietrix F., Ussi A. E. *Et Al*. (2019). The Fundamental Characteristics Of A Translational Scientist. Acs Pharmacol. Transl. Sci., 2: 213–216.
- Hall Je (2002). The Promise of Translational Physiology. Am. J. Physiol. (Lung Cell Mol Physiol.) 283: L235–L236.
- Homer-Vanniasinkam S. and Janice Tsui_J. (2012). The Continuing Challenges of Translational Research: Clinician-Scientists' Perspective. Cardiol. Res. Pract. 2012: 246710.
- Jaja S.I., Ogungbemi S.O., Kehinde M.O. And Anigbogu C.N. (2016). Supplementation With L-Arginine

- Stabilizes Plasma Arginine And Nitric Oxide Metabolites, Suppresses Elevated Liver Enzymes And Peroxidation In Sick Cell Anaemia. *Pathophysiol.* 23: 81–85.
- Jaja S.I., Oludemi B. F. And Renner J. K. (2002). The Effect Of Load-Bearing Either On The Back Or On The Chest On Oxygen Consumption And Energy Expenditure. *Niger J. Health And Biomedical Sci.* 1(1): 41-44.
- Liebman M.N. And Marincola F.M. (2012). Expanding The Perspective Of Translational Medicine: The Value Of Observational Data. *J. Translational Med.* 10:61.
- Marincola Fm. (2003). Translational Medicine: A Two-Way Road. *J. Translational Med. Medicine* 1(1):1-2.
- Olatunji G., Emmanuel K., Osaghae O.W., Timilehin I., Aderinto N., Abdulbasit M.O. (2023). Enhancing Clinical and Translational Research In Africa: A Comprehensive Exploration Of Challenges And Opportunities For Advancement. *J. Clin. Translational Res.* 9(5): 357-368
- Piel F.B., Patil A.P., Howes R.E., Nyangiri O.A., Gething P.E., Dewi M., *Et Al.* (2013). Global Epidemiology of Sick Cell Haemoglobin In Neonates: A Contemporary Geosttistical Model-Based Map And Population Estimates. *Lancet* 381:142–151.
- Sadeghi A., Taherifard E., Ebrahimi N.D., Rafiei E., Hadianfard F. And Taherifard E (2023). Effects Of L-Arginine Supplementation in Patients with Sick Cell Disease: A Systematic Review And Meta-Analysis Of Clinical Trials. *Health Sci. Rep.* 6: E1167.
- Saka W.A., Anigbogu C.N., Kehinde M.O. And Jaja S.I. (2023). L-Arginine Supplementation Enhanced Expression Of Glucose Transporter (Glut 1) In Sick Cell Anaemia Subjects In The Steady State. *Cur. Res. Physiol.* 6: 100096.
- Salami A., Kanmod K.K. (2021). Translational Medical Research In Nigeria: Challenges, Prospects And Recommendations For The Future. *Euro. J. Translational Clin. Med.* 4(1):71-78.
- Salisu, T. F., Okpuzor, J. E. And Jaja, S. I. (2019). Identification, Characterization And Quantification Of Chemical Compounds In Selected Edible Wild Leafy Vegetables. *Ife J. Sci.* 21(1): 215–227.
- Salisu T.F., Ottu B.O., Oloyo A.K., Okpuzor J.E. And Jaja S.I. (2022). Assessment Of Wild African Vegetables On Cardiac Functions, Antioxidants And Blood Lipid Profile In A Rat Model Of Myocardial Infarction. *Fuw Trends In Sci. & Tech. J.* 7(1): 070–082.
- Seals Dr (2013). Translational Physiology: From Molecules to Public Health. *J. Physiol.* 591: 3457–3469.
- Tchernia D. And Diallo G. (2002). Sick Cell Disease in Africa. *Cur. Opinion in Hematol.* 9(2):111-116.
- Van Der Laan A.L. and Boenink M. (2012). Beyond Bench and Bedside: Disentangling The Concept of Translational Research. *Health Care Analysis* 23; 32-49.

Review article

Review of Immune-Metabolic Studies and Re-purposed Treatments of Nigerian COVID-19 Patients: A Pointer to Mild, Gender- and Age-Based Status of Admitted Patients

*Arinola O.G.¹, Onifade A.A.¹, Adigun K.², Oshingbesan M.B.³.

¹Department of Immunology, University of Ibadan, Nigeria

²Department of Family Planning, University College Hospital, Ibadan, Nigeria

³Norfolk and Suffolk NHS Foundation Trust, Mariner House, 43 Handford Road, Ipswich, IP1 2GA

Summary: When Severe Acute Respiratory Human Coronavirus 2 (SARS-hCoV 2) infection began in December 2019, detailed knowledge about the virus was lacking. This included non-availability of anti-viral treatment or vaccine, no knowledge of virus-human interaction, and lack of prognostic factors for stages of illness among others. A publication in Nigerian Journal of Physiological Sciences (2020). 35: 20-25 titled “Immune Responses During Human Coronavirus Infection: Suggestions For Future Studies” adduced investigations into immune parameters of COVID-19 patients so as to throw more light on the immunopathogenesis of SAR-CoV-2 infection, in order to create avenue for the development of vaccines or herd immunity. This present publication is a review of studies carried out on COVID-19 patients in one Infectious Diseases Center (I.D.C), Ibadan, Nigeria as a response to the gaps in knowledge raised in above mentioned publication. Cumulatively, immune-metabolic studies from this IDC revealed mild, age- and sex-dependent status of COVID-19 in patients admitted into this center. Thus, explaining the basis for the effectiveness of adopted re-purposed drugs (chloroquine or hydroxychloroquine, zinc, vitamins C and D and or antibiotics), physiotherapy and nutritional support used for the management of admitted COVID-19 patients. Also, this paper vindicated that inflammation was heightened during SARS-CoV 2 infection; therefore therapeutic interventions to control the inflammatory processes, oxidative stress, antibodies against structural and non-structural proteins or blocks receptor sites were proposed. In addition, development of herd immunity and efficacy of COVID-19 vaccines (Astrazeneca and Moderna) were elucidated in general population. However, study to determine host genetic factors in hCoV infection was lacking. This review concluded that interdisciplinary collaborative approach will be useful in the management of future emerging or re-emerging infection.

Keywords: Antibodies, Herd-immunity, Inflammation, Phagocytes, Re-purposed drugs, Vaccines.

*Authors for correspondence: drarinolaog64@yahoo.com, Tel: +234-80-23451520

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INTRODUCTION

The causative agent (SARS-Cov 2) of coronavirus disease 2019 (COVID-19) was discovered in January 2020 to be betacoronavirus in the same subgenus as Severe Acute Respiratory Syndrome Coronavirus 2 (WHO, 2020). For its invasion, it was established that SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) as entry receptors on host cells (Hoffmann *et al.*, 2020). The host innate immune system detects dsRNA (a Pathogen-Associated Molecular Patterns, PAMPs) of CoV using Pattern Recognition Receptors (Toll-like receptors, mannose receptor, scavenger receptor; mannose-binding lectin and C-reactive protein), follow by NF- κ B activation which promotes the synthesis of type I IFNs and other proinflammatory cytokines (Schneider *et al.*, 2014). IL-1, IL-6, IL-8, TNF- β and MCP-1 produced by infected cells in response to CoV infection attract, retain and activate

lymphocytes and leukocytes to the site of infection (Edem and Arinola 2015). This stage is associated with high fever, hypoxemia and progression to pneumonia-like symptoms despite progressive decline in virus titers (Peiris *et al.*, 2003). Two immune lung cells (alveolar macrophages and nerve/airway associated-macrophages), four types of blood immune cells (antibody-secreting cells, follicular helper T cells, activated CD4⁺ T cells and CD8⁺ T cells) and immunoglobulin M and IgG antibodies activate immunity to CoV (Fehr and Perlman, 2015). Macrophages present viral peptides to B- and T- lymphocytes leading to adaptive immunity. For easy understanding, this review is separated into the following sections, viz: humoral cellular and humoral adaptive immune responses-, metabolic responses- and repurposed treatment responses- of COVID-19 patients one I.D.C, Nigeria.

Humoral innate immune responses of COVID-19 patients in Ibadan: The first study (Arinola *et al.*, 2021c)

on hospitalised COVID-19 patients in an IDC, Ibadan, Nigeria provided demographic features of these patients. 58% of these patients were between age 18 and 35 years, 48.1% were employees of private establishments, and 64.1% were males. High proportion (84.3%) of the patients spent less than 14 days on admission with no mortality as at June 2020. The findings of this study emphasised the importance of gender and age on therapeutic strategies.

Transmission of SARS-CoV 2 was through aerosols; breathe through nostrils though ocular infection was also assumed (Arinola, 2020). To prevent nasal SARS-CoV 2 entry, the wearing of nose-mask was instituted during COVID-19 pandemic. Following successful SARS-CoV 2 entry into the nostrils of host, innate factors such as nasal cilia filtration, mucus trapping action and muco-ciliary movements prevented further spread. The ingestion of SARS-CoV 2 into the gut was likelihood, which caused the detection of CoV 2 particles in the faeces. Moreover, SARS-CoV 2 might also be ejected through sneezing causing further viral spread. The failure of these initial host innate functions to block the entrance of the virus resulted to attachment and invasion of host cell by CoV 2, followed by viral replication and production of genomic dsRNA in the cytoplasm. Recognition and sensitisation of Pattern Recognition Receptors (especially TLR-3) by dsRNA and NF- κ B activation promoted the synthesis type I IFNs and other proinflammatory cytokines (Jiang *et al.*, 2020). Also, on the adaptive aspect of immune response, the processed CoV 2 in the macrophages and its peptide presented to T cells leading to T cell activation and production of cytokines, complement factors, acute phase proteins and respiratory burst factors (McKechnie and Blish, 2020).

Respiratory burst function, which is the release of reactive oxygen species from leucocytes, is one of the innate immune mechanisms to prevent the establishment of intracellular pathogens in the host. A study (Arinola *et al.*, 2021f) in Nigerian COVID-19 patients showed that mean plasma levels of nitric oxide (NO), myeloperoxidase (MPO) and hydrogen peroxide (H₂O₂) were significantly decreased while SOD was significantly increased in COVID-19 patients at admission compared with control. In male COVID-19 patients above 40yrs of age, the level of MPO was significantly increased compared with MPO level in female COVID-19 patients below 40yrs of age. The study concluded that respiratory burst function as an innate immune response was involved in the immunopathogenicity of SARS-CoV 2; and that MPO might explain differential prevalence of COVID-19 among gender and age groups.

Another study (Akinwumi *et al.*, 2021) determined the prognostic value of some cellular inflammatory cells and their indices in relation to duration of hospital admission, gender, and age of Nigerian COVID-19 patients. This longitudinal and case-control study determined blood cell components (total white blood cells (TWBC), neutrophil, lymphocyte, monocyte, and platelet) and inflammatory indices (neutrophil lymphocyte ratio [NLR], lymphocyte monocyte ratio [LMR], platelet lymphocyte ratio [PLR], derived NLR [DNLR], and systemic immune inflammatory index [SII]. The mean platelet count and PLR were significantly lower while TWBC counts were significantly increased in COVID-19 patients compared with control. The mean neutrophil count, PLR, and SII were significantly

lower while mean lymphocyte count was significantly higher in COVID-19 patients aged <40 years compared with patients aged $\geq$ 40 years. This study concluded that inflammatory response is a phenomenon in COVID-19 patients especially in patients $\geq$ 40 years of age and that this inflammation persist till discharge, though gender had no influence on cellular inflammatory indices of COVID-19 patients.

Acute phase proteins are sensitive systemic markers of inflammation, nutritional status and tissue damage while COVID-19 is an inflammatory disease with tissue damaging effects. It was thus assumed that high sensitive C-reactive protein (a marker of inflammation), albumin and prealbumin (markers of nutritional status) could be prognostic of COVID-19 disease among Nigerian patients (Arinola *et al.*, 2022). High sensitive C-reactive protein (hsC-RP) level was significantly higher in newly admitted COVID-19 patients compared with discharged COVID-19 patients or COVID-19 free control ($p < 0.05$). The mean values of plasma hs C-RP, albumin and prealbumin in most COVID-19 patients (89%, 100% and 91% respectively) were within normal reference ranges. hsC-RP were significantly increased in newly admitted COVID-19 patients who are females, above 40years of age and in COVID-19 patients with hospital admission above 10days ($p < 0.05$ in each case). The study also found higher plasma hsCRP level in female than male COVID-19 patients or COVID-19 patients above 40years compared with those below 40years of age. Therefore, plasma hs C-RP level was concluded to be a useful prognostic marker of COVID-19 and that low grade inflammation due to host C-RP and viral dsRNA existed in COVID-19 patients. The high hsCRP in COVID-19 patients was implicated in the cardiovascular episode of poorly managed COVID-19 patients.

It was documented that co-infection of malaria and COVID-19 existed and that malaria reduced the severity of COVID-19 (Anyanwu 2021), but the mechanisms of malaria parasitaemia on SARS-CoV 2 susceptibility and severity remained unclear. A study (Arinola *et al.*, 2021b) that determined the cellular and humoral factors of oxidative burst (aspect of innate immunity) in COVID-19 patients in IDC, Ibadan, Nigeria reported that the level of plasma nitric oxide was significantly higher in co-infection of COVID-19 and malaria (CoV+P) compared with COVID-19 without malaria (CoV-P) or control. Myeloperoxidase enzyme activity was significantly higher in CoV+P compared with CoV-P. Blood cell counts, hydrogen peroxide, superoxide dismutase and catalase were similar in all groups. In summary, raised levels of NO and MPO were produced by phagocytic cells in CoV+P patients. Taken together, the findings indicated that raised NO in CoV+P might have regulated the adverse cardiovascular episodes of MPO in CoV+P patients. Another longitudinal study (Akinwumi *et al.*, 2023) using biochemical nutritional markers (albumin, prealbumin and total cholesterol) and nutritional indices [Controlling Nutritional Status (CONUT) score and Prognostic Nutritional Index (PNI)] classified Nigerian COVID-19 patients at IDC, Ibadan as mild. Also, in this study, it was found that most COVID-19 patients (89.5%) were well-nourished based on Prognostic Nutritional Index. Reports indicated that immune complexes (ICs) might play active roles in COVID-19 immunopathology (Ankerhold *et al.*, 2022; Kolb *et al.*, 2023), but the changes in circulating

IC (CIC) level during the course of COVID-19 was not determined. Plasma level of CIC in Nigerian adults with SARS-CoV-2 infection was followed up from diagnosis till discharged from IDC, Ibadan (Arinola *et al.*, 2021c). The mean CIC level was higher in COVID-19 patients at diagnosis compared with the control. However, there was no significant difference in the mean plasma levels of CIC at discharge compared with the level at diagnosis in COVID-19 patients. It was concluded from this study that the plasma level of CIC might be involved in the immunopathology of COVID-19; COVID-19 was thus concluded to be an immune complex disease.

The presence of cytokine storm syndrome as a characteristic of COVID-19 patients was exploited in the formulation of management strategies of these patients. A study investigated the effect of repurposed management on serum Th1 pro-inflammation cytokine (IFN- γ) and Th2 anti-inflammation cytokine (IL-4) in Nigerian COVID-19 patients (Arinola *et al.*, 2023a). The mean values of IFN- γ and IL-4 were significantly higher in COVID-19 patients at admission compared with discharged COVID-19 patients whereas IFN- γ :IL-4 ratio was significantly higher in discharged COVID-19 patients compared with admitted COVID-19 patients. Significantly higher proportion of COVID-19 patients at discharge had IFN- γ within the normal reference ranges compared with COVID-19 patients at admission whereas the proportions of COVID-19 patients at discharge and COVID-19 patients at admission having IL-4 within the normal reference ranges were the same. The study concluded that the repurposed treatment suppressed pro-inflammation cytokine (IFN- γ) in most discharged COVID-19 patients.

Humoral adaptive immune responses of COVID-19 patients in Ibadan: Dearth of specific therapy for COVID-19 despite its pandemicity called for a clear understanding of antibody based protective immunity following natural infection with SARS-CoV 2. This was expected to give knowledge on effectiveness of vaccines or convalescent plasma against SARS-CoV-2 and possibility of development of herd immunity. To provide these informations, the following studies were carried out on COVID-19 patients from IDC, Ibadan, Nigeria. Arinola (2021) reported that CovIgG was positive in none (0)% and 20% of COVID-19 patients at admission and at discharge respectively while CovIgM was positive in 54% and 69% of COVID-19 patients at admission and at discharged respectively. The level of CovIgG was significantly higher in COVID-19 patients at discharge compared with the level at admission while the level of CovIgM was insignificantly reduced in COVID-19 patients at discharge compared with the level at admission. The data strongly suggested that plasma from recovered patients might give opportunity for the development of effective antibody-based therapies to treat COVID-19 patients. Using cassette system lateral flow immunoassay (Arinola *et al.*, 2021d), anti-SARS 2 Cov-IgG and -IgM antibodies were not detected in all COVID-19 patients either at diagnosis or discharge, 7.1% COVID-19 patients were detected for the combination of anti-CovIgG and anti-CovIgM antibodies at discharge. Also, anti-SARS 2 -IgG and -IgM antibodies were more prevalent in COVID-19 patients at discharge than those newly diagnosed, thus not all plasma from all COVID-19 patients should be

consider for plasma therapy. This result had implications for vaccine development, vaccine efficacy and serological surveys.

The importance of IgA in mucosal immune system might open a novel approach in therapeutic settings against COVID-19. A study (Fasasi *et al.*, 2023) among Nigerian COVID-19 patients showed that the mean value of serum IgA was significantly increased in COVID-19 patients at admission or COVID-19 patients at discharge compared with the level in corresponding control. The mean value of serum IgA was similar in COVID-19 patients at admission compared with COVID-19 patients at discharge, thus concluded that serum IgA could be a useful biomarker to differentiate patients with COVID-9 from un-infected controls. The major importance was that IgA was protective in COVID-19 patients and might be one of the neutralizing antibodies stimulated by COVID-19 vaccines.

Neutralizing antibodies (Nabs) play critical roles in blocking viral infections, viral clearance during acute infection or controlling disease progression during chronic phase. CovIgG which was found to have neutralizing activity (Wu *et al.*, 2020) and Nabs in the plasma of convalescent COVID-19 patients were successfully used in the passive antibody therapy to treat severe cases of SARS-CoV-2 infection (Duan *et al.*, 2020). But, the rapidly declining CoV specific antibodies from 6 months created challenge on the duration of COVID-19 antibody based vaccine and convalescence plasma therapy (Wu *et al.*, 2020).

Apart from the pandemicity of COVID-19, availability and accessibility to molecular testing was also limited. Thus, to provide epidemiologic data and to contain outbreaks, a number of diagnostic testing methods and kits were developed for COVID-19 (Yang *et al.*, 2020). The widely use diagnosis test for COVID-19 was the nucleic acid amplification tests (NAATs) which detected the presence of the viral genome and current infection (Jacofsky *et al.*, 2020). Another testing method was the serological test which detected the anti-SARS-CoV 2 antibodies (mainly IgG, IgM and IgA) produced by the immune system, but this was not suitable for very early diagnosis as there is a time lag (6 – 7 days) for antibody production to viral antigens (Jacofsky *et al.*, 2020). There were a number of point-of-care tests for SARS-CoV 2 diagnosis however; the reliability of these test kits was poorly documented. The sensitivity and specificity of two serological test kits for COVID-19 diagnosis in Nigeria using a commercially available Rapid Diagnostic Test (RDT) and enzyme linked immunosorbent assay (ELISA) was carried out (Arinola *et al.*, 2021d). The sensitivity and specificity of the test kits were determined using ROC while the Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), Diagnostic Odds Ratio (DOR) and accuracy were calculated as appropriate. Twenty eight (59.6%) of the study participants had positive PCR result. ELISA and RDT identified 20 (42.6%) and 13 (27.7%) participants respectively as having COVID-19. ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT while the RDT had a better specificity performance than ELISA. The proportion of participants with positive SARS-CoV-2 IgM antibody identified using ELISA was significantly higher compared with RDT. In

contrast, the proportion of participants with positive SARS-CoV-2 IgG antibody identified using RDT was significantly higher compared with ELISA. The importance of this study was that ELISA had a better sensitivity for SARS-CoV-2 Spike-protein specific antibodies than the RDT. However, combination of RDT and ELISA for SARS-CoV-2 antibodies might be useful for population COVID-19 screening.

Metabolic responses of COVID-19 patients in Ibadan:

ACE is an integral receptor on almost all organs (Du *et al.*, 2020) and it is needed for SARS-CoV 2 host invasion, therefore its presence served as the basis for multi-organ dysfunction of COVID-19 patients. Apart from multi-organ presence of ACE 2, the presence of viral RNA in the blood coupled with viral replication in the intestine made it plausible that SARS-CoV 2 could enter the portal circulation to reach the liver. The attempt by hepatic Kupffer cells to clear the virus initiated an inflammatory response. Also inflammatory mediators from the intestine enter the portal system and sinusoids (Jose and Manuel, 2020) which might have been responsible for the observed liver- and renal- dysfunctions in Nigerian COVID-19 patients (Arinola *et al.*, 2021e; 2023, Alonge and Arinola, 2022). Therefore, full understanding of metabolic pathways of SARS-CoV 2 could facilitate therapeutic insights for COVID-19 management. High proportions of COVID-19 patients had values of the liver (59%-96%) and renal (43%-97%) function test parameters within the normal reference intervals. Similarly, high proportions of COVID-19 patients had values of lipid profile (71%-86%) within the normal reference intervals. The infrequent alteration in lipid metabolism as well as liver and renal functions suggests mild COVID-19. It could be concluded from this study that renal and liver dysfunctions as well as lipid dysmetabolism are uncommon in patients with mild COVID-19.

Angiotensin Converting Enzyme-2 is known to affect renal functions, vasoconstriction and fluid homeostasis (Du *et al.*, 2020). The receptor of this ACE also attaches to SARS-CoV 2, thus the impact of SARS-CoV-2 infection on renal function parameters is worth investigating. Plasma obtained from whole blood samples Nigerian COVID-19 patients were analysed for albumin, urea, creatinine, Na, K, Cl and HCO₃. It was observed that 57.1%, 37.8%, 32.7%, 28.1%, 18.7%, 17.8% and 3.4% of newly diagnosed COVID-19 patients had values of Cl, creatinine, albumin, Na, K, HCO₃ and urea respectively outside the reference ranges. While 43.3%, 4.7%, 2.5%, 2.5%, 2.0%, 1.7% and 1.0% of COVID-19 patients had values of Cl, creatinine, Na, K, albumin, Urea and HCO₃ respectively above the reference ranges. Of all admitted patients, 33.1%, 30.7%, 25.6%, 16.8%, 16.3%, 13.8% and 1.7% had creatinine, albumin, Na, HCO₃, K, Cl and urea values respectively below reference ranges. The changes in some renal function parameters of newly diagnosed COVID-19 patients portend that renal failure is possible in poorly managed COVID-19 patients and this has immunopathologic implications.

LDH is an enzyme involved in energy production by conversion of lactate to pyruvate and it is present in almost all body cells with highest levels in heart, liver, lungs, muscles, kidneys and blood cells (Lu *et al.*, 2018). LDH is also an indicator of acute or chronic tissue damage, and

inflammation (Sepulveda, 2013). There is close relation between LDH organ distribution, hypoxia, immune dysregulation with COVID-19 pathogenesis, it is thus reasonable to determine the level of serum LDH as prognostic factor of COVID-19. Changes in LDH activity from admission through discharge were determined in Nigerian SARS-CoV-2 patients with a view to determining its prognostic properties (Onifade *et al.*, 2023). The mean serum LDH activity was significantly higher in COVID-19 patients at admission and at discharge compared with the controls. However, the mean LDH activity was slightly lower in COVID-19 patients at discharge compared with the activity at admission. It was concluded from the study that LDH activity is elevated in COVID-19 patients and changes in its activity during the course of COVID-19 management could provide clinical information on patient's response to therapy. The values of activities of LDH in our COVID-19 patients within normal reference range indicated that majority of COVID-19 patients in our study had mild COVID-19. This observation corroborates previous reports which classified COVID-19 in patients from the same IDC as mild (Alonge *et al.*, 2022, Arinola *et al.*, 2022). This study suggested the possible use of LDH inhibitors as metabolic checkpoint in the management of COVID-19 patients.

Clinical trial of IDO inhibitor or uses of micro-nutrient supplements during management of diseases is commonly done without having adequate basis for the practise. Tryptophan (Trp) is an essential amino acid needed for T-lymphocyte function, and indoleamine-2,3-dioxygenase (IDO) is a potent immunoregulatory molecule that catalyses the rate-limiting step of Trp degradation in the kynurenine (Kyn) pathway (Ünüvar *et al.*, 2019). Mean IDO activity in COVID-19 patients was significantly higher compared with corresponding control. The data suggested that IDO inhibitor might be necessary in COVID-19.

Repurposed treatment responses of COVID-19 patients

in Ibadan: SARS-CoV-2 shares almost 80% of the genome with SARS-CoV (Lu *et al.*, 2020). Therefore, the immune-metabolic attributes of earlier covid viruses were assumed similar to those of SARS-CoV-2 making it easy to formulate a repurposed treatment for COVID-19, which included chloroquine or hydroxychloroquine, zinc, vitamins C and D and or antibiotic(s) designed by the Case Management Team of the Oyo State COVID-19 Task Force (Alonge *et al.*, 2022). Physiotherapy and nutritional support for these patients were also included. See Table below. This repurposed drug treatment also arose as a result of no known specific drugs or vaccine developed to manage or prevent COVID-19 (Wu *et al.*, 2020).

National Institute of Health (NIH) and Food and Drug Administration (FDA) recommended, remdesivir and convalescent blood products as promising potentials for COVID-19 treatment apart from the uses of chloroquine, hydroxychloroquine, favipiravir, ivermectin, and colchicines (Hossen *et al.*, 2020). Usage of albendazole antihelminthic drug (Onifade and Arinola, 2020), plant extracts with antiviral potentials (Attah *et al.*, 2021) and protease inhibitors (Olubiyi *et al.* 2020) were also proposed.

Table 1:

Medication used by COVID-19 patients at an IDC, Ibadan, Nigeria.

Medications	Groups
	COVID-19 Patients
Vitamin D (1,000 iu)	1,000 iu twice daily for 3 weeks
Vitamin C (1,000 mg)	1,000 mg twice daily for 3 weeks
Zn (20mg)	100 mg daily for 3 weeks
Azithromycin	500 mg daily for 3 days
Hydroxychloroquine	400 mg on day 1 and 200 mg daily for 3 more days
Chloroquine (As an alternative to Hydroxychloroquine)	500 mg on day 1 and 250 mg daily for 3 more days

Santos *et al* (2020) reported that therapeutics for COVID-19 includes camostatmesylate, remdesivir, favipiravir, tocilizumab, baricitinib, convalescent plasma, and humanized monoclonal antibodies. Other treatment options for COVID-19 included therapeutics targeting the spike protein attachment to human cells (Yang *et al.*, 2020). The repurposing of chloroquine and hydroxychloroquine for treatment of SARS-CoV-2 infection was premised on the anti-inflammatory properties of these antimalarial which may be beneficial to the patients if there is underlying malaria parasitaemia and also to reduce pre-empted occurrence of inflammatory cytokine storm which is associated with SARS-Cov-2 infection (Yang *et al.*, 2020). Meo *et al* (2020) proposed that chloroquine and hydroxychloroquine are beneficial in the management of COVID-19 through inhibition of the receptor binding by the virus, inhibition of membrane fusion by the virus and immune modulation leading to a decrease in cytokine release. The use of vitamin D in the management of COVID-19 patients hinged on the autocrine function (Arinola and Edem, 2020; Rahamon *et al.*, 2021); vitamin C has immunomodulatory and antioxidant capacity, zinc regulates the number and function of immune cells (macrophages, neutrophils, dendritic cells, mast cells, T cells and B cells) (Haase and Rink, 2007). Immunomodulatory and anti-inflammatory functions of vitamin D, vitamin C and Zn made them appropriately supportive in the management of COVID-19 patients. Also, in this environment (Nigeria), a randomised trial (ClinicalTrials.gov ID: NCT04459286) was conducted by Fowotade *et al.*, (2022) where mild to moderate COVID-19 patients received standard of care (SoC) or SoC plus a 14-day course of nitazoxanide (1,000 mg b.i.d.) and atazanavir/ritonavir (300/100 mg od) and followed through day 28. Nitazoxanide co-administered with atazanavir/ritonavir was found to be safe but not better than standard of care in treating COVID-19 in this trial. The HIV protease inhibitor, atazanavir (boosted with ritonavir), blocks pro-inflammatory cytokine production and has been shown to inhibit the major protease enzyme required for viral polyprotein processing during coronavirus replication (Fintelman-Rodrigues *et al.*, 2020). Additionally, tizoxanide is inactivated by glucuronidation and atazanavir is a well-known inhibitor (Zhang *et al.*, 2005). Hence, atazanavir was expected to enhance tizoxanide exposure when used in combination with nitazoxanide.

Conclusion: Knowledge gained from these studies could be applied against emerging or re-emerging diseases.

Further Studies: Future studies should develop strategies that differentiate immune responses during vaccination from active SARS-CoV 2 infection, host genetics during COVID-19 and sequelae of post-COVID-19 experience.

Limitations: (a). As a monocentric review, it may be difficult to generalise results to other regions of varying epidemiological characteristics, (b). data on pre-admission herbal medicines or other drugs used as self-medication before developing COVID-19 were not available, (c). most of the further studies can only be done on archived samples.

REFERENCES

- Akinwumi JA, Edem VF, Arinola GO. (2021). Cellular inflammatory indices in hospitalised Nigerian COVID-19 patients. *Journal Health Science Research*. 6(2): 19-26.
- Akinwumi J, Edem VF, Arinola, OG. (2023). Calculated Nutritional Indices in Symptomatic Hospitalized Nigerian COVID-19 Patients. *Nigerian Journal of Physiological Sciences*, 38(1), 17–22. <https://doi.org/10.54548/njps.v38i1.4>.
- Alonge O and Arinola G. (2022). Haematological Changes and Metabolic Alterations in SARS-CoV-2 Infected Patients Hospitalised At an Infectious Diseases Center, Ibadan, Nigeria. *Eur.J. Clin. And Exp. Med*. 20(4): 383-390.
- Alonge O, Fowotade A, Bamidele F, Omotosho T, Aboluwoye, M, Olulana S, Fashina N, Famuyiwa F, Eegunjobi A, Arinola G. (2022). Clinical Outcome Of Corona Virus Disease-19 Patients In An Infectious Disease Center, Olodo, Ibadan, Oyo State, Nigeria. *Clinical Medicine Insights*, 32; 287–296.
- Ankerhold J, Giese S, Kolb P, Maul-Pavicic A, Voll RE, Göppert N, Ciminski K, Kreutz C, Lother A, Salzer U, Bildl W, Welsink T, Morgenthaler NG, Grawitz AB, Emmerich F, Steinmann D, Huzly D, Schwemmler M, Hengel H, Falcone V. (2022). Circulating multimeric immune complexes contribute to immunopathology in COVID-19. *Nat Commun*. Sep 26;13(1):5654. doi: 10.1038/s41467-022-32867-z. PMID: 36163132; PMCID: PMC9513013.
- Anyanwu MU. (2021). The association between malaria prevalence and COVID-19 mortality. *BMC Infect Dis*. Sep 19;21(1):975. doi: 10.1186/s12879-021-06701-8. PMID: 34538242; PMCID: PMC8449890.
- Arinola G.O, Alonge T.O, Edem F.V, Fowotade A, Fashina O.A, Akinbola O.I, Akinbile S.A, Eegunjobi A.O, Bello M.D, Fagbemi O.S, Salami F, Johnson J.O, Oke A.C, Famuyiwa O.I, Olaoti A.J, Olaniyan O.A, Ishola C.O. (2021e). Changes in Selected Renal Function Parameters of Newly Admitted COVID-19 Patients from One Infectious Diseases Center in Ibadan, Nigeria: Indication for Immunopathology. *Nig. J. Physiol. Sci*. 36: 11 – 15.

- Arinola GO, Onifade AA, Edem VF, Rahamon SK, Alonge OT. (2023a). Immunometabolic Profile of Nigerian COVID-19 Patients. *Journal of Health Science and Medical Research*, [S.l.], p. e2023945, mar. 2023. ISSN 2630-0559. Available at: <<https://www.jhsmr.org/index.php/jhsmr/article/view/945>>.
- Arinola GO, Edem FV, Fashina OA, Olaniyan OA, Alonge TO. (2021b). Cellular and Humoral Factors of Oxidative Burst in COVID-19 Patients with Malaria Parasitemia. *Annals of Epidemiology & Public Health*. 4(1): 1060-1065.
- Arinola GO, Edem VF, Alonge TO. (2021a). Respiratory Burst Functions in COVID-19 Nigerian Patients. *Journal of Basic and Applied Research in Biomedicine*. 7(1): 24-28.
- Arinola GO, O.A. Fashina, O.C. Oluyomi Ishola, O.I. Akinbola, S.A. Akinbile, A.O. Eegunjobi, M.D. Bello, F.V. Edem, S.K. Rahamon, O.I. Famuyiwa, A.J. Olaoti, O.A. Olaniyan, A.C. Oke, A. Fowotade, O. Abimbola, J.O. Johnson, O.S. Fagbemi, Funmi Salami, T.O. Alonge (2021c). Demographic attributes of COVID-19 patients in an infectious disease center of Nigeria. *Afr. Journal of Clinical and Experimental Microbiology*. 22(1): 21-27.
- Arinola GO, Onifade AA, Adigun K, Oshingbesan MB. (2023a). Serum levels of IFN- γ and IL-4 in hospitalised COVID-19 patients – evidence of reduced cytokine storm in discharged patients. *Eur J Clin Exp Med*. 21(4):750–755. doi: 10.15584/ejcem.2023.4.14.
- Arinola GO, Onifade AA, Edem VF and Yaqub SA (2021d). Detection of anti SARS-COV 2 specific -IgG and -IgM antibodies in COVID-19 patients using rapid screening immunochromatographic cassettes. *Annals of Epidemiology and Public Health*. 4(1): 1059.
- Arinola O.G (2020). Immune Responses During Human Coronavirus Infection: Suggestions For Future Studies: *Nigerian Journal of Physiological Sciences*. 35: 20-25.
- Arinola O.G and Edem V.F (2020). Antioxidant Vitamins are correlated with Different Aspects of Phagocytic Processes in Healthy Nigerians: Benefit as Supplements during Antimicrobial Treatment. *Sudan Journal of Medical Sciences*. 15 (3): 223–234. DOI 10.18502/sjms.v15i3.7253.
- Arinola O.G, Edem V.F, Rahamon S.K, Yaqub S.A, Fashina A.O and Alonge T.O. (2020). SARS-CoV-2 Infection Screening Using Two Serological Testing Methods. *Nigerian. J. Physiol. Sci*. 35 (December 2020): 117 – 121.
- Arinola OG. (2021). Serum Levels of Anti-Corona Virus Specific-IgG and -IgM Antibodies in COVID-19 Patients At Admission and At Discharge. *European Journal of Clinical and Experimental Medicine*. 19(1): 5-9.
- Arinola OG, Rahamon SK, Edem VF, Yaqub SA, Fashina OA, Alonge TO. (2021g). Elevated plasma level of circulating immune complexes in Nigerian adults infected with Severe Acute Respiratory Syndrome Corona Virus-2. *Arch. Basic and Applied Medicine*. 9: 69-72
- Arinola OG, Rahamon SK, Edem VF, Yaqub SA, Fashina OA, Alonge TO. (2021). Elevated plasma level of circulating immune complexes in Nigerian adults infected with Severe Acute Respiratory Syndrome Corona Virus-2. *Arch. Basic and Applied Medicine*. 9: 69-72
- Arinola, G. O., Edem, F. V., & Alonge, T. O. (2022). Levels of Plasma C-reactive protein, Albumin and Pre-Albumin in Nigerian COVID-19 Patients. *Annals of Medical Research*, 29(1), 0046–0051. Retrieved from <https://annalsmedres.org/index.php/aomr/article/view/4021>
- Arinola GO, Abdullahi I, Rahamon SK. et al. (2023). Activities of plasma indoleamine-2, 3-dioxygenase (IDO) enzyme in Nigerian patients with lung diseases: basis for tryptophan supplementation or IDO inhibitor use. *Egypt J Bronchol* 17, 2 <https://doi.org/10.1186/s43168-022-00174-2>
- Attah AF, Fagbemi AA, Olubiyi O, Dada-Adegbola H, Oluwadotun A, Elujoba A and Babalola CP. (2021). Therapeutic Potentials of Antiviral Plants Used in Traditional African Medicine With COVID-19 in Focus: A Nigerian Perspective. *Front. Pharmacol*. 12:596855. doi: 10.3389/fphar.2021.596855
- Du M., et al. (2020). Multi-omics evaluation of gastrointestinal and other clinical characteristics of SARS-CoV-2 and COVID-19. *Gastroenterology*. 158:2298–2301. doi: 10.1053/j.gastro.2020.03.045. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
- Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. (2020). Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci USA*. 117: 9490–9496. doi: 10.1073/pnas.2004168117
- Edem VF and Arinola OG (2015). Leucocyte migration and intracellular killing in newly diagnosed pulmonary tuberculosis patients and during anti-tuberculosis chemotherapy. *Annals of Global Health*. 81(5): 669-674.
- Fasasi ZB, Abdullahi I, Kehinde A, Bakare AA & Arinola G. O. (2023). Assessment of Serum Levels of Immunoglobulin A (IgA) Class in Patients with COVID-19 and Asthma. *Asian Journal of Immunology*. 6(1), 102–111. Retrieved from <https://journalaji.com/index.php/AJI/article/view/101>.
- Fehr AR and Perlman S (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. *Methods Mol Biol*. 1282: 1–23. doi: 10.1007/978-1-4939-2438-7_1.
- Fintelman-Rodrigues N, Sacramento CQ, Lima CR, Silva DFS, Ferreira AC, Mattos M, et al. (2020). Atazanavir inhibits sars-cov-2 replication and pro-inflammatory cytokine production. *bioRxiv* [Preprint]. doi: 10.1101/2020.04.04.020925
- Fowotade A, Bamidele F, Egbetola B, Fagbamigbe AF, Adeagbo BA, Adefuye BO, Olagunoye A, Ojo TO, Adebisi AO, Olagunju OI, Ladipo OT, Akinloye A, Onayade A, Bolaji OO, Rannard S, Happi C, Owen A and Olagunju A (2022). A randomized, open-label trial of combined nitazoxanide and atazanavir/ritonavir for mild to moderate COVID-19. *Front. Med*. 9:956123. doi: 10.3389/fmed.2022.956123
- Haase H and L. Rink. (2007). Signal Transduction in Monocytes: The Role of Zinc Ions. *Biometals*. 20(3): 579–585.
- Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 181: 1-10.
- Jacofsky D, Jacofsky EM, Jacofsky M. (2020). Understanding Antibody Testing for COVID-19. *J Arthroplasty*. Jul;35(7S):S74-S81. doi: 10.1016/j.arth.2020.04.055. Epub 2020 Apr 27. PMID: 32389405; PMCID: PMC7184973.
- Jiang Y, Zhao T, Zhou X, Xiang Y, Gutierrez-Castrellon P, Ma X. (2020). Inflammatory pathways in COVID-19: Mechanism and therapeutic interventions. *MedComm* (2020). 2022 Aug 1;3(3):e154. doi: 10.1002/mco2.154. PMID: 35923762; PMCID: PMC9340488.
- Jose RJ, Manuel A. (2020). COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med*. 2020; 8: e46-e47.
- Kolb P, Giese S, Voll RE, Hengel H, Falcone V. (2023). Immune complexes as culprits of immunopathology in severe COVID-19. *Med Microbiol Immunol*. Apr;212(2):185-191. doi: 10.1007/s00430-022-00743-8.
- Lu J, Wei Z, Jiang H et al. (2018). Lactate dehydrogenase is associated with 28-day mortality in patients with sepsis: a retrospective observational study. *J Surg Res*. 228:314–321. doi:10.1016/j.jss.2018.03.035.

- Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, *et al.* (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 395:565–74. doi: 10.1016/S0140-6736(20)30251-8
- McKechnie JL, Blish CA. (2020). The Innate Immune System: Fighting on the Front Lines or Fanning the Flames of COVID-19? *Cell Host Microbe*. Jun 10;27(6):863-869. doi: 10.1016/j.chom.2020.05.009. Epub 2020 May 20. PMID: 32464098; PMCID: PMC7237895.
- Meo S.A, D.C. Klonoff, J. Akram. (2020). Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19. *European Review for Medical and Pharmacological Sciences*. 24:4539-4547
- Olaleye DO, Adewale Victor Opayele, Hyacinth Chukwuebuka Egbuna, Adedayo Omotayo Faneye, Babatunde Adebisi Olusola, Timothy Segun, Elisabeth Chibuzo Odeh, Magbagbeola David Dairo, Adeniyi Francis Fagbamigbe, Musa Adamu Sarki, Maryam Aminu, Ademola Johnson Ajuwon, Ganiyu Arinola, Chris Ezeora Achi, Sunday Aremu Omilabu, Georgina Njideka Odaibo. (2023) Molecular detection of SARS-CoV-2 infection in three geo-political zones of Nigeria: a cross-sectional study. *Pan African Medical Journal*. 44:146. [doi: 10.11604/pamj.2023.44.146.37400]
- Olaleye DO, Aminu M, Olusola BA, Segun TO, Faneye AO, Opayele AV, Odeh E, Sarki M, Dairo MD, Fagbamigbe AF, Egbuna HC, Ajuwon AJ, Omilabu SA, Achi CE, Odaibo GN and Arinola OG (2022). High Seroprevalence of SARS-CoV-2 Antibody before Covid-19 Vaccination in Nigerian Communities. *International Archives of Public Health and Community Medicine*. DOI:10.23937/2643-4512/1710083. ISSN: 2643-4512.
- Olubiyi OO, Olagunju M, Keutmann M, Loschwitz J, Strodel B. (2020). High Throughput Virtual Screening to Discover Inhibitors of the Main Protease of the Coronavirus SARS-CoV-2. *Molecules*. 25. 3193. <https://doi.org/10.3390/molecules25143193>
- Onifade AA, Rahamon SK and Arinola OG. (2023a). Serum lactate dehydrogenase activity in COVID-19 patients at admission and discharge from an isolation center: Suggestion as metabolic checkpoint. *Arch. Bas. App. Med.*11(1):32-36.
- Onifade AA and Arinola OG (2020). Albendazole reduces serum levels of inflammatory cytokines: Potential strategy for the management of cytokine storm”. *Saudi Journal of Biomedical Research*. 5 (11): 320-324. DOI : 10.36348/sjbr.2020.v05i11.006
- Onifade AA, Fowotade A, Rahamon SK, Edem VF, Yaqub SA, Akande OK, Arinola OG. (2023b). Seroprevalence of anti-SARS-CoV-2 specific antibodies in vaccinated and vaccine naïve adult Nigerians. *PLoS ONE* 18(1): e0280276. <https://doi.org/10.1371/journal.pone.0280276>
- Peiris JS *et al.* (2003). Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet*. 361(9371):1767–1772
- Rahamon SK, Arinola OG, Charles-Davies MA, *et al.* (2021). Serum levels of vitamin D and tumour necrosis factor-alpha in adults with metabolic syndrome. *Eur J Clin Exp Med*. 19(4):306–312. doi: 10.15584/ejcem.2021.4.3.
- Schneider WM, Chevillotte MD, Rice CM. (2014). Interferon gamma stimulated genes: a complex web of host defenses. *Annu Rev Immunol*. 32: 513- 545.
- Sepulveda J. (2013). Challenges in Routine Clinical Chemistry Analysis: Proteins and Enzymes. Editor(s): A. Dasgupta, J. L. Sepulveda, Chapter 9, Accurate Results in the Clinical Laboratory, *Elsevier*,2013:131-148.
- Ünütvar S, Erge D, Kılıçarslan B, Bağ H, Girgin G and Baydar T. (2019). Neopterin Levels and Indoleamine 2,3-Dioxygenase Activity as Biomarkers of Immune System Activation and Childhood Allergic Diseases. *Ann Lab Med*. 39(3): 284-290. <https://doi.org/10.3343/alm.2019.39.3.284>.
- WHO Director-General's opening remarks at the media briefing on COVID-19 – 11 March 2020. World Health Organization. 11 March 2020. Retrieved 11 March 2020.
- Wu F., *et al.* (2020). Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *MedRxiv Preprint*, <https://doi.org/10.1101/2020.1103.1130.20047365> (2020).
- Wu R, Wang L, Kuo HD, Shannar A, Peter R, Chou PJ, Li S, Hudlikar R, Liu X, Liu Z, Poiani GJ, Amorosa L, Brunetti L, Kong AN. (2020). An Update on Current Therapeutic Drugs Treating COVID-19. *Curr Pharmacol Rep*. 11:1-15. doi: 10.1007/s40495-020-00216-
- Yang L, Shasha Liu, Jinyan Liu, Zhixin Zhang, Xiaochun Wan, Bo Huang, Youhai Chen and Yi Zhang. (2020) COVID-19: immunopathogenesis and Immunotherapeutics. *Signal Transduction and Targeted Therapy*. 5;128:1-8.
- Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, *et al.* (2020). Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. *medRxiv [preprint]* 2020.02.11.20021493. doi: 10.1101/2020.02.11.20021493 CrossRef Full Text | Google Scholar
- Zhang D, Chando TJ, Everett DW, Patten CJ, Dehal SS, Humphreys WG. (2005). In vitro inhibition of udp glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab Dispos*. 33:1729–39. doi: 10.1124/dmd.105.005447.

Full length Research Article

Trends of Twin Births in Kogi State, North-Central Nigeria

***Bakare A.A.^{1, +}, Peter D.A.¹, Coker M.M.¹ and Fagbenro O.S.¹**

Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria

Summary: Globally, Nigeria has the highest rate of twin births. However, within the country, there are variations in twinning rates. Report on the twinning rate in Kogi state, north-central Nigeria, is scarce. A 30-year retrospective study of the occurrence of twin births between 1991 and 2020 in Kogi state, north-central Nigeria was conducted. Data were obtained from six hospitals in the state: Federal Medical Centre Lokoja, General Hospital Mopa-Muro, Zonal Hospital Kabba, Specialist Hospital Obangede, General Hospital Okene, and Prince Abubakar Audu University Teaching Hospital Anyigba. The data were analysed monthly, quarterly, and yearly for twinning frequency using descriptive statistics and one-way ANOVA. There were 1783 twin deliveries during the study period, accounting for 2.60% of all deliveries. The annual occurrence of twinning ranged from 20.9% at the Federal Medical Centre Lokoja to 28.5% at the Zonal Hospital Okene resulting in 25.1% as the mean frequency of twin births for the study period in Kogi state. There is no significant difference ($p>0.05$) between the variations in the twinning rate. The fluctuation in the frequency of twinning in Kogi state could be due to increased use of oral contraceptives, urbanisation, socioeconomic factors, and a decrease in family size and reproductive age.

Keywords: Twin birth, Kogi state, Frequency of twinning, Twins in Nigeria.

*Authors for correspondence (Current address): Department of Biology, National University of Lesotho, P.O. Roma 180, Maseru, Lesotho. E-mail: adebakar19@yahoo.com, adekunle.bakare@ui.edu.ng; Tel.: +266 560 05829

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INTRODUCTION

Twin birth, a pregnancy event that leads to the delivery of two babies or foetuses can either result in live birth or stillbirth (Lu *et al.*, 2013). While twins are revered and celebrated in certain communities, they are considered unusual in others (Smits and Monden, 2011). Twin births occur in two forms: identical (monozygotic; MZ) and fraternal (dizygotic; DZ). The constancy in the rate of occurrence of MZ twins (approximately 3-5 twins per 1000) has confirmed its spontaneity and randomisation (Nwankwo *et al.*, 2013). Variation in DZ twinning is greatly affected by numerous factors which include genetics, family history, geographical location, maternal age, maternal weight and height, lifestyle, diet, socio-economic status, family size, and Assisted Reproductive Technology (ART) (Obiechina *et al.*, 2011; Nilsen *et al.*, 2016; Akinseye *et al.*, 2019).

The continual use of oral contraception has been found to lead to a high incidence of twinning due to high rebound gonadotropin secretion (Oyaromade *et al.*, 2021). In developed nations, ART has been a major cause of the increase in the frequency of twinning in recent years (Monden *et al.*, 2021). Twin birth rate is lowest among young mothers (19 - 24 years) and increases with an increase in maternal age (25 - 34 years) which then drops sharply because of a decline in the pool of growing follicle-stimulating hormone (FSH) concentration (Sunday-Adeoye *et al.*, 2008; Akinseye *et al.*, 2019). Geographical variation

has significantly influenced the twinning rate globally with some countries having very high rates of twin births and others having extremely low rates. Though, Africa is reported to be the highest twin producer in the early period of the 21st century (Sunday-Adeoye *et al.*, 2008), the rate of twinning has rapidly increased in Asia which has a nearly equal frequency of twinning rate with Africa (Monden *et al.*, 2021).

In Nigeria, the highest occurrence of multiple births including twins, triplets, and quadruplets is predominantly found in Igbo-Ora, a rural town in Oyo State in the Southwestern part of the country (Akinseye *et al.*, 2019). The high rate of twin births in this community which celebrates multiple births annually is traced to a set of diets believed to contain phyto-hormones that trigger the production of FSH (Akinboro *et al.*, 2008; Olotu *et al.*, 2023). The incidence and frequency of twinning from different parts of the six geopolitical zones of Nigeria have been reported (Akinboro *et al.*, 2008; Bakare *et al.*, 2011; Adewale *et al.*, 2018; Gabriel *et al.*, 2020; Wekere *et al.*, 2021; Igbodike *et al.*, 2024). To date, there has been no report on the occurrence and frequency of twin births in Kogi State, one of the six north-central states in Nigeria where there are very few reports. This study was undertaken to determine the incidence and frequency of twin births in Kogi state, Nigeria. We believe that the data obtained will assist policymakers in the demographic analysis of the state.

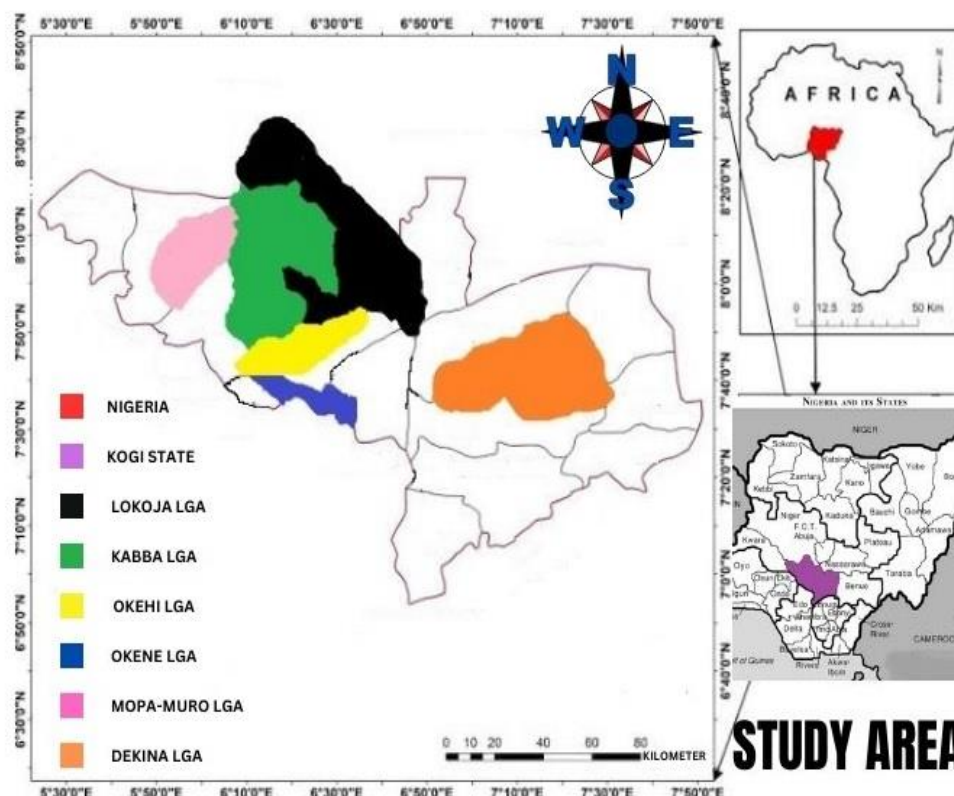


Figure 1:

Kogi State, Nigeria showing the Local Government areas of the Hospitals used for data collection

MATERIALS AND METHODS

Study Location: Six Hospitals in Kogi State, north-central Nigeria (Fig. 1): a Federal Government Hospital, and five State Government Hospitals including the State University Teaching Hospital were used for the data collection. These Hospitals serve as primary, secondary, and tertiary health centers, and respectively are, Federal Medical Centre Lokoja (established on the 9th of November 1999), Zonal Hospital Kabba (established on the 1st of January 1989), Zonal Hospital Okene (established on the 1st of January 1960), General Hospital Mopa-Muro (established on the 1st of January 1999), Specialist Hospital Obangede (established on the 10th of January 1982), and Prince Abubakar Audu University Teaching Hospital Anyigba (established on the 29th of February 2011). The cluster sampling method was used to divide Kogi State into the senatorial districts (Kogi East, Kogi Central, and Kogi West) after which the hospitals were selected in each of the three districts using a purposive sampling technique. The selected government hospitals are the major hospitals with more patronage by pregnant women compared to the private hospitals most of which are not more than 10 years in operation. Kogi state (Fig. 1) located at a latitude of 7° 45' 0N and longitude of 6° 45' 0E was created on the 27th of August, 1991. It is one of the six north-central states (Benue, Kogi, Kwara, Nasarawa, Niger, and Plateau) and ranks as the 13th largest state in Nigeria. The state shares boundaries with nine other states (Niger, and Nasarawa to the North; Ondo, Ekiti, and Kwara to the West; Enugu, Anambra, and Edo to the South; and Benue to the East) and the Federal Capital Territory. The total land mass of the State is 29,833km and the population projection in 2016 was 4,473,500 (National Bureau of Statistics,

Nigeria; official Gazette (FGP 71/52007/2500(OL24)). There are three major ethnic groups (Okun, Ebira, and Igala) and several minor ethnic groups including the Ogori, Bassa-Nge, Bassa-Kwomu, Koton, Nupe, Oworo, and Gwari in Kogi State.

Methodology: Data on childbirth records between 1991 and 2020 were extracted from the maternity sections and the central records departments of the various hospitals. Due to inadequate record keeping, and industrial strike action by workers, records for some months and years are missing from the data on each hospital. The approval for data collection was obtained from the Chief Medical Director of the Hospital Management Board at Lokoja in Kogi State (the body that oversees the state government-owned hospitals in the State), and from the Medical Director of the Federal Medical Centre at Lokoja. This study was executed according to the ethical guidelines of World Medical Association (2022) Helsinki Declaration, and the individual consent for this retrospective analysis was waived.

Statistical analysis: The Microsoft Office Excel® 2023 was used for data analysis. The trend and incidence of twinning were analysed using descriptive statistics to determine monthly, quarterly, and annual rates and frequencies. The number of single births, twin births, and total births were denoted by "x", "y", and "z" respectively. The number of single births for every twin delivery (x/y) was determined. In every 1000 deliveries, the number of twin births was calculated as

$$\frac{\text{Twin deliveries (y)}}{\text{Total deliveries (z)}} \times 1000$$

RESULTS

Tables 1 - 6 present the annual occurrence of twin births at the Federal Medical Centre Lokoja, Zonal Hospital Kabba, Zonal Hospital Okene, General Hospital Mopa-Muro, Specialist Hospital Obangede, and Prince Abubakar Audu University Teaching Hospital Anyigba in Kogi state respectively. There was no regular pattern of seasonal change in twin births from the records of the six hospitals. At the Federal Medical Centre Lokoja (Kogi West Senatorial District), there were 45.2‰ births and 1.9‰ births as the highest and lowest twin births in 2002 and 2009 respectively (Table 1). There was no record of twin birth in 2000. There were 20.9 twin births on average per 1000 total deliveries, and 40.4 single births for every twin delivery. Figure 2 shows the time plot of the occurrence of twinning for the 240-month study period. Thirteen peaks were observed during the period occurring in January, March, April, May, June, July, August, September, October, and December with frequencies of 1, 2, 2, 1, 1, 1, 1, 2, 1 and 1 respectively. The peaks occurred randomly in the months of the year but were mostly seen in March, April, and September.

From the records obtained from 1993 to 2020 at Zonal Hospital Kabba, Kogi West Senatorial District (Table 2), the highest and lowest twin birth rates of 57.9‰ and 10.0‰ were obtained in 1996 and 1993 respectively. During the study period, there were 28.0 twin births per 1000 deliveries and 34.8 single births for every twin delivery on average. The time plot of the occurrence of twinning for the period (300 months) is presented in Figure 3. Thirteen peaks were observed in January, February, March, April, May, June, July, August, September, and November with frequencies of

1, 4, 1, 3, 1, 3, 1, 1, 2, and 1 respectively. The peaks occurred randomly but were mostly seen in February, April, and September.

Table 1:

Annual incidence of twin births at the Federal Medical Centre, Lokoja between 2000 and 2019

Year	Single del. (x)	Twin del. (y)	Total del. (z)	Single for each twin delivery (x/y)	Twin births per 1000 delivery
2000	566	0	566	-	-
2001	819	25	844	32.8	29.6
2002	1394	66	1460	21.1	45.2
2003	1575	63	1638	25.0	38.5
2004	1908	62	1970	30.8	31.5
2005	1901	46	1947	41.3	23.6
2006	1674	73	1747	22.9	41.8
2007	2225	64	2289	34.8	28.0
2008	2264	68	2332	33.3	29.2
2009	1572	3	1575	524.0	1.9
2010	1371	8	1379	171.4	5.8
2011	2254	6	2260	375.7	2.7
2012	2015	30	2045	67.2	14.7
2013	2385	71	2456	33.6	28.9
2014	1475	56	1531	26.3	36.6
2015	1352	36	1388	37.6	25.9
2016	1178	29	1207	40.6	24.0
2017	382	18	400	21.2	45.0
2018	207	8	215	25.9	37.2
2019	1495	11	1506	135.9	7.3
Total	30012	743	30755	40.4	20.9

Key: del. = delivery(ies)

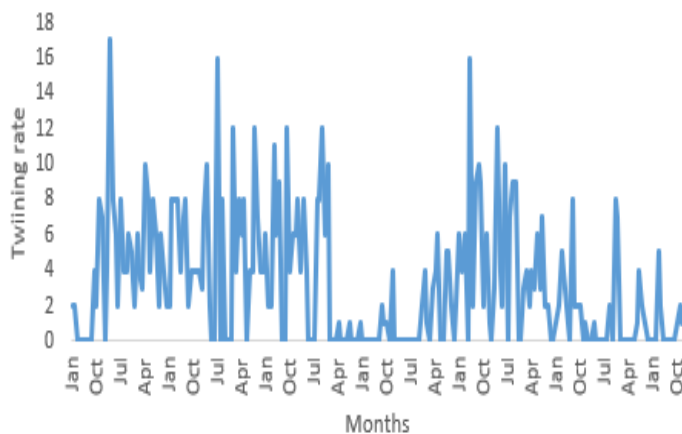


Figure 2:

Time plot of the incidence of twinning recorded at the Federal Medical Centre, Lokoja for 20 years (2000 - 2019).

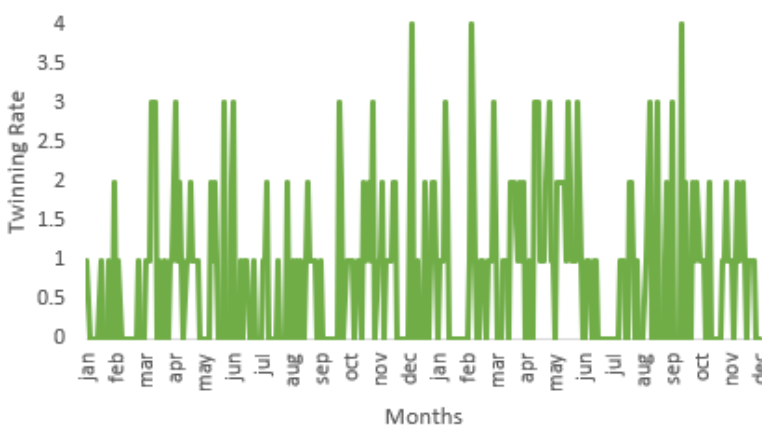


Figure 3:

Time plot of the incidence of twinning recorded at the Zonal Hospital Kabba for 25 years (1993 - 2020, there was no record for 2007-2009).

Annual incidence of twin births at the Zonal Hospital, Kabba
between 1993 and 2020

Key: *del.* = *delivery(ies)*

The graph displays the twinning rates over a 36-month period. The y-axis, labeled 'Twinning Rates', has a scale from 0 to 16 in increments of 2. The x-axis, labeled 'Months', consists of 36 'Jan' labels. The orange line represents the data, showing significant fluctuations. Notable peaks occur at month 25 (rate ~16.5), month 10 (rate ~8), and month 30 (rate ~8). The rate remains at 0 for a period between month 18 and month 20.

Annual incidence of twin births at the Zonal Hospital Okene
between 1991 and 2020

Key: del. = delivery(ies)

Figure 4:

Time plot of the incidence of twinning at the Zonal Hospital, Okene for 22 years (1991 - 2020).

Table 4:

Annual incidence of twin births at the General Hospital, Mopa-Muro Kogi State between 1999 and 2020

Years	Single del. (x)	Twin del. (y)	Total del. (z)	Single for each twin delivery (x/y)	Twin birth per 1000 delivery
1999	55	0	55	-	-
2000	67	1	68	67.0	14.7
2001	96	1	97	96.0	10.3
2002	84	0	84	-	-
2003	94	2	96	47.0	20.8
2004	130	3	133	43.3	22.6
2005	126	1	127	126.0	7.9
2006	130	3	133	43.3	22.6
2007	143	3	146	47.7	20.5
2008	152	6	158	25.3	38.0
2009	112	3	115	37.3	26.1
2010	140	2	142	70.0	14.1
2011	127	4	131	31.8	30.5
2012	141	4	145	35.3	27.6
2013	118	2	120	59.0	16.7
2014	128	4	132	32.0	30.3
2015	49	0	49	-	-
2016	77	4	81	19.3	49.4
2017	51	2	53	25.5	37.7
2018	63	1	64	63.0	15.6
2019	76	1	77	76.0	13.0
2020	69	0	69	-	-
Total	2228	47	2275	36.3	24.9

Key: del. = delivery(ies)

Table 5:

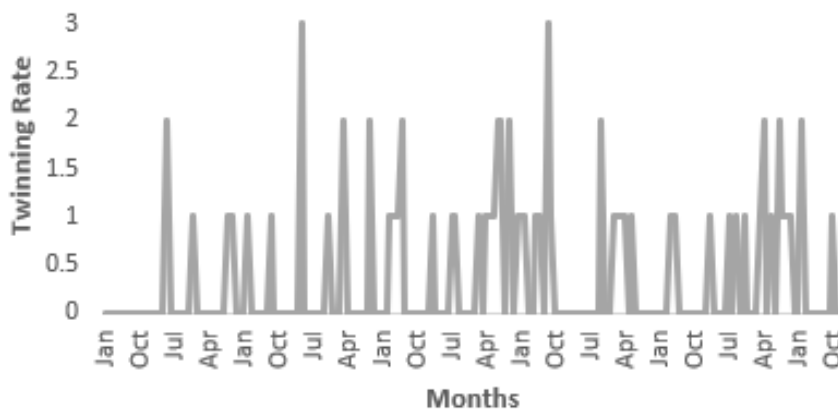
Annual incidence of twin birth at the Specialist Hospital Obangede, Okehi Local Government area of Kogi State between 2003 and 2020

Years	Single del. (x)	Twin del. (y)	Total del. (z)	Single for each twin delivery (x/Y)	Twin birth per 1000 delivery
2003	89	0	89	-	-
2005	124	3	127	41.3	23.6
2006	195	2	197	97.5	10.2
2007	55	2	57	27.5	35.1
2008	111	4	115	27.8	34.8
2009	106	4	110	26.5	36.4
2010	142	5	147	28.4	34.0
2011	78	0	78	-	-
2012	167	3	170	55.7	17.6
2013	237	11	248	21.5	44.4
2014	265	8	273	33.1	29.3
2015	75	2	77	37.5	26.0
2016	132	5	137	26.4	36.5
2017	115	2	117	57.5	17.1
2018	172	4	176	43.0	22.7
2019	212	9	221	23.6	40.7
2020	191	3	194	63.7	15.5
Total	2466	67	2533	36.8	26.5

Key: del. = delivery(ies)

**Figure 5:**

Time plot of the incidence of twinning at the General Hospital, Mopa-Muro for 22 years (1999 - 2020).

**Figure 6:**

Time plot of the incidence of twinning at the Specialist Hospital Obangede for 17 years (2003 - 2020).



Figure 7:
Time plot of the incidence of twinning at the Prince Abubakar Audu University Teaching Hospital Anyigba for 16 years (2005 - 2020).

The mean of twin births per 1000 deliveries was 26.5 births, and the mean of single births for each twin delivery was 36.8. The Time plot of the incidence of twinning for the 204 months study period shows twelve peaks in February, March, April, May, June, July, August, October, and November with frequencies of 1, 1, 2, 1, 1, 1, 2, 2 and 1, respectively (Figure 6). The peaks occurred in March and August.

At the Prince Abubakar Audu University Teaching Hospital, Anyigba (Kogi East Senatorial District, Table 6) the highest and lowest twin birth rates of 65.8‰ and 12.3‰ were obtained in 2006 and 2020 respectively. The mean of twin births per 1000 deliveries pooled for the period was 21.7 births, and the mean of single births for each twin delivery was 38.6. A time plot of the occurrence of twinning for the 192 months of the study period is presented in Figure 7. Nine peaks were observed in May, June September, October, November, and December with frequencies 3, 2, 1, 2, and 1 respectively. The peaks occurred in November. Cumulatively, there were 68,654 deliveries in the six hospitals during the study period. The mean of twin births per 1000 deliveries was 25.1 births, and the meanof single births for each twin delivery was 37.5 (Table 7).

Table 6:
Annual frequency of twin births at the Prince Abubakar Audu University Teaching Hospital between 2005 and 2020

Years	Single del (x)	Twin del (y)	Total del (z)	Single for each twin delivery (x/y)	Twin birth per 1000 delivery
2005	65	0	65		0.0
2006	142	10	152	14.2	65.8
2007	153	2	155	76.5	12.9
2008	149	3	152	49.7	19.7
2009	183	4	187	45.8	21.4
2010	264	8	272	33.0	29.4
2011	190	6	196	31.7	30.6
2012	359	17	376	21.1	45.2
2013	442	9	451	49.1	20.0
2014	358	6	364	59.7	16.5
2015	234	7	241	33.4	29.0
2016	228	3	231	76.0	13.0
2017	108	2	110	54.0	18.2
2018	194	8	202	24.3	39.6
2019	317	7	324	45.3	21.6
2020	321	4	325	80.3	12.3
Total	3707	96	3803	38.6	21.7

Table 7:
Frequency of twin births in Kogi State, north-central Nigeria from 1991 to 2020

Years	Single del. (x)	Twin del. (y)	Total del. (z)	Single for each twin delivery (x/y)	Twin birth per 1000 delivery
FMCL	30012	743	30755	40.4	20.9
ZHK	8345	240	8585	34.8	28.0
ZHO	20117	590	20707	34.1	28.5
GHMM	2228	47	2271	47.4	24.9
SHO	2466	67	2533	36.8	26.5
PAAUTHA	3707	96	3803	38.6	21.7
Total	66875	1783	68654	37.5	25.1

FMCL - Federal Medical Centre Lokoja, ZHK - Zonal Hospital Kabba, ZHO - Zonal Hospital Okene, GHMM - General Hospital Mopa-Muro, SHO - Specialist Hospital Obangede, PAAUTH - Prince Abubakar Audu University Teaching Hospital Anyigba

DISCUSSION

This is a 30-year retrospective evaluation of the prevalence of twin births in Kogi State, north-central Nigeria. Five of the six hospitals including a University teaching hospital are owned by the state government, while the sixth is owned by the federal government. Throughout the period analysed herein, there was no specific pattern in twin birth incidence per 1000 deliveries. The overall mean occurrence of twin births for the study period in Kogi state is 25.1‰, and it ranged from 20.9‰ at the Federal Medical Centre, Lokoja to 28.5‰ at the Zonal Hospital, Okene. This frequency falls within the range of values obtained in Nigeria. It is higher than the reported value of 23.2‰ in Kano, 22‰ in Zamfara, 17.3‰ in Katsina, and 8.5‰ in Sokoto northern Nigeria (Attah *et al.*, 2014; Onankpa and Nauzo, 2014; Lawal *et al.*, 2019; Oyaromade *et al.*, 2021), and Ile-Ife in southern Nigeria (Igbodike *et al.*, 2024), but lower than the reported value of 35.1‰ (Fakeye, 1986) and 37.4‰ (Iyiola *et al.*, 2013) in Kwara State, 32.5‰ in Abuja (Akaba *et al.*, 2013); 39.7‰ among Hausa women (Rehan and Tafida, 1980), 35.9‰ in Ibadan (Bakare *et al.*, 2011), 28‰ in Jos (Aisien *et al.* 2000), 68.1‰ in Oyo, 46.5‰ in Osun, 29.5‰ Delta,

and 30.0‰ in Ekiti (Akinboro *et al.*, 2008; Igberase *et al.*, 2008; Iyiola *et al.*, 2013; Akinseye *et al.*, 2019).

The value obtained herein appears to lie between values from the other parts of Nigeria. Kogi state which is centrally located in Nigeria shares boundaries with 10 states from the north, east, west, and southern parts of the country, and is easily accessible to migrants from these zones and encourages inter-tribal marriage. Migration and inter-ethnic mixing are factors known to modify twinning rates (Knox and Morley, 1960). The type of food consumed may also be an important factor in the incidence of twinning in Kogi state. Olotu *et al.* (2023) investigated some food products including yam, cassava, okra leaf, beans, grains, maca root, pineapple, dairy products, fruit, and vegetables. It revealed the presence of bioactive ingredients like FSH and luteinizing hormones, phytoestrogen, gonadotropin, insulin-like protein, enzyme bromelain, zinc, and folic acid which are believed to induce multiple pregnancies. These are major farm produce that serve as the main diets consumed by the people of Kogi state who are also mostly farmers.

The high incidence of twinning in Okene (28.5‰) may be due to genetics, family size, and maternal weight. In this town, siblings from the same parent are seen to be carriers of multiple pregnancies. As a developing town, there is limited access to information on family planning; hence, an average family comprises 4 to 6 children. The huge family size accounts for the large population size in the zone. The fertility level of the reproductive age of both men and women is also high. This can be attributed to a low level of exposure to environmental toxins in a town with no or little industrial activities. Kabba and Mopa-Muro are located in the western part of Kogi state and the indigenous dwellers are believed to have originated from the southwestern part of Nigeria (the region with the highest twin birth rate) as they share some cultural beliefs including language, dressing, marriage patterns, and food. The value obtained for Kabba (28.0‰) is relatively close to 30.0‰ obtained in Ekiti a neighboring southwestern state (Akinboro *et al.* 2008), while that of Mopa-Muro (24.9‰) is lower. This discrepancy can be accounted for by the population size, choice, and preference of health centers. Kabba is more developed than Mopa-Muro and the hospital serves as the major health centre for the zone. Moreover, the presence of qualified personnel, the number of services rendered, and the availability of necessary health equipment attract health seekers. The values from the records at the specialist Hospital in Obangede (26.5‰) are also relatively lower than those from Okene which is within the same zone. The variation can be attributed to the population size and urbanisation.

The incidence of twin births at the Federal Medical Centre, Lokoja is the lowest. The hospital is located in Lokoja, Kogi state capital with an estimated 2024 population of 885,882 (United Nations World Urbanization Prospects, 2018). The low value from this location could be attributed to urbanisation, socioeconomic factors, lifestyle, low family size, environment, and diet (Gan *et al.*, 2007). There is an interplay between these factors, as urban dwellers are more exposed to processed foods and junk due to their busy lifestyles and have little or no access to naturally prepared food. Exposure to toxins from industrial and commercial

activities is also common in the city. A limitation of this study is the number of birth records accessible for analysis. In Nigeria, 37.3% of births occur in hospitals (Sule and Madugu, 2004). It is a common trend in the study area to use local methods for child deliveries and these are not recorded in hospitals or birth registries.

In conclusion, in this study, we have presented data for the first time on the occurrence of twin births from different parts of Kogi state, north-central Nigeria using records of births from six different hospitals located in the different zones of Kogi state. Data analysis shows a high rate of twin births in Kogi state and is consistent with values from other parts of Nigeria. The central location of the state in Nigeria probably influenced the data, it has paved the way for migration and a wide range of inter-tribal relationships between the indigenes and people from other parts of the country. The twinning rate can also be attributed to urbanisation, geographical location, genetics, family size, coital rate, and ART.

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REFERENCES

- Adewale, F.B., Ashimi, A. and Oyewopo, O.A. (2018). Trends of multiple births at federal medical centre Bida, Northcentral Nigeria. *Trop J Obstet Gynaecol* 35:68-72. DOI: 10.4103/TJOG.TJOG_57_17.
- Aisien, A.O., Olarewaju, R.S. and Imade, G.E. (2000). Twins in Jos Nigeria: A seven-year retrospective study. *Med Sci Monit* 6:945-50.
- Akaba, G.O., Agida, T.E., Onafowokan, O., Offiong, R.A., and Adewole, N.D. (2013). Review of twin pregnancies in a tertiary hospital in Abuja, Nigeria. *Health Popul Nutr* 31(2):272-277. doi: 10.3329/jhpn.v31i2.16392.
- Akinboro, A., Azeez, M.A., and Bakare, A.A. (2008). Frequency of twinning in southwest Nigeria. *Ind J Hum Genet* 14(2): 41–47.
- Akinseye, K.M., Anifowoshe, A.T., Owolodun, O.A., Aina, O.M. and Iyiola, O.A. (2019). Frequency of Twinning in Nigeria: A Review. *Man J Sci* 12:78–88.
- Attah, R.A, Mohammed Z. and Gobir, M.A. (2014). A review of twin deliveries in Aminu Kano Teaching Hospital, North-West Nigeria. *Nig J Basic Clin Sci* 11(1):3-7.
- Bakare, A.A., Elegbede, E.O. and Osowole, O.I. (2011). Trends of human sex ratio at birth and winning rate in Ibadan, South-Western Nigeria. *The Zoologists* 9:47-56. <https://www.ajol.info/index.php/tzool/article/view/73128>.
- Fakeye, O. 1986. Perinatal factors in twin mortality in Nigeria. *Int J Gynaecol Obstet* 24:309-14.
- Gabriel, A.M., Abasi, B.E. and Okupa, O.D. (2020). The prevalence and the outcome of twin pregnancies in a South-South Nigerian tertiary hospital. *J Med Sci Clin Res* 08 (01): 1-8: <https://dx.doi.org/10.18535/jmscr/v8i1.01>
- Gan, J.P., Wu, Z.H., Tu, Z.M. and Zheng, J. (2007). The comparison of twinning rates between urban and rural areas in China. *Twin Res Hum Genet* 10:633-637.

- Igbodike, E.P., Ijarotimi, O.A., Ubom, A.E., Eleje, G.U., God'swill, C.C., Okpala BC, Nwaogu, N.L., Ajenifuja, K.O., Ikechebelu, J.I., Loto, O.M., Onwudiegwu, U., and Eke, A.C. (2023). Trends and Outcomes of Twin Births in Southwest Nigeria: A 14-Year Retrospective Cohort Study. *Explor Res Hypothesis Med* 9(1): 15-24., doi: 10.14218/ERHM.2023.00039
- Igberase, G.O., Ebeigbe, P.N., and Bock-Oruma, A. (2008). Twinning rate in a rural mission tertiary hospital in the Niger Delta, Nigeria. *J Obstet Gynaecol* 28(6): 586–589.
- Iyiola, O.A., Oyeyemi, F.B., Raheem, U.A., and Mark, F.O. (2013). Frequency of twinning in Kwara state, North-Central Nigeria. *Egyptian J Med Hum Genet* 14:29-35.
- Knox, G. and Morley, D. (1960). Twinning in Yoruba women. *J Obstet Gynaecol Br Emp* 67:981-984.
- Lawal, A.M., Atabo-Peter, O.D. and Abdurrahman, A. (2019). Twin pregnancies at federal medical centre Katsina: A 5 year review. *Trop J Obstet Gynaecol* 36:183-188.
- Lu, X., Zhang, J., Liu, Y., Wang, T., Lu, Y. and Li, Z. (2013). Epidemiology of Twin Births in Southeast China: 1993–2005. *Twin Res Hum Genet* 16(2):608–613.
- Monden, C., Pison, G. and Smits, J. (2021). Twin peaks: more twinning in humans than ever before. *J Hum Reprod* <https://doi.org/10.1093/humrep/deab029>
- National Bureau of Statistics, Federal Republic of Nigeria Official Gazette. (2007). 2006 Population Census (FGP 71/52007/2,500 (OL 24): Legal notice on publication of the details of the breakdown of the National and State Provisional Totals 2006 census.(www.nigerianstat.gov.ng).
- Nilsen, T., Magnus, P. and Ørstavik, R. (2016). Maternal body composition in relation to twinning. *Norsk Epidemiologi*. 26 (1-2): 77-81. doi:10.5324/nje.v26i1-2.2019
- Nwankwo, T.O., Aniebue, U.U., Ezenkwele, E. and Nwafor, M.I. (2013). Pregnancy outcome and factors affecting vaginal delivery of twins at University of Nigeria Teaching Hospital, Enugu. *Nig J Clin Pract* 16, 490–495.
- Obiechina, N.J., Okolie, V.E., Eleje, G.U., Okechukwu, Z.C and Ane, O.A. (2011). Twin versus singleton pregnancies: the incidence, pregnancy complications, and obstetric outcomes in a Nigerian tertiary hospital. *Internat J Wom Health* 3:227–230. <http://dx.doi.org/10.2147/IJWH.S22059>.
- Olotu, P.N., Olotu, I.A., Yakubu, N.S., Onuche, E.U., Famojuro, T.I., Datok, T., Ogira, J.O. and Ohemu, T.L. (2023). A review of Nigerian plants and their bioactive constituents that increase chances of multiple pregnancies. *J Pharmacog. Phytochem* <https://doi.org/10.22271/phyto.2023.v12.i4c.14685>
- Onankpa, B.O., and Nauzo, A.M. (2014). Incidence and outcome of neonates of multi-fetal gestations admitted to a tertiary hospital in northwestern Nigeria. *Internat J Health Sci Res* 4(7), 76–82.
- Oyaromade, A., Muhammad, B.A. and Omigbodun, A. (2021). A three-year review of twin deliveries in a specialist hospital in North-Western Nigeria. *Internat J Front Life Sci Res* 01(01):034–039. <https://doi.org/10.53294/ijflsr.2021.1.1.0034>.
- Rehan, N. and Tafida, D.S. 1980. Multiple births in Hausa women. *Br J Obstet Gynaecol* 87:997-1004.
- Smits, J. and Monden, C. (2011). Twinning across the Developing World. *PLoS ONE* 6(9): e25239. doi:10.1371/journal.pone.0025239.
- Sule, S.T., and Madugu, H.N. (2004). Sex ratio at birth in Zaria. *Ann Hum Biol* 31:258-62.
- Sunday-Adeoye, I., Twomey, E.D. and Egwuatu, V.E. (2008). A 20-year review of twin births at Mater Misericordiae Hospital, Afikpo, South Eastern Nigeria. *Nig J Clin Pract* 11(13):231-234.
- United Nations World Urbanization Prospects. (2018). Department of Economic and Social Affairs of the United Nations 2018 Revision of World Urbanization Prospects, <https://population.un.org/wup/>
- Wekere, F.C.C., John, D.H., Clement-Wekere, G.A.F. and Iwo-Amah, R.S. (2021). Prevalence, trend and outcome of twin pregnancy in Rivers State University Teaching Hospital, Southern Nigeria. *Int J Reprod Contracept Obstet Gynecol* 10:2571-2577. DOI: 10.18203/2320-1770.ijrcog20212641.
- World Medical Association. (2022). WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/#:~:text=Medical%20research%20involving%20human%20subjects%20must%20be%20conducted%20only%20by,or%20other%20health%20care%20professional>

Full length Research Article

Mean Values, Normal Limits and Sex Differences of Heart Rate, QT Interval, QTc, PR Interval and QRS Duration among Young Adults of Yoruba Ethnicity

***Awoyinka D.I. and Ogunlade O.**

Department of Physiological Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

Summary: An electrocardiogram (ECG) is important for non-invasive cardiovascular health assessment. This study was carried out to determine the mean values, normal limits, and sex differences of selected electrocardiographic variables in young adults of Yoruba ethnicity. One thousand healthy volunteers (500 males and 500 females) aged 18 to 40 years participated in the study. Ethical approval was obtained from the Institute of Public Health of the University. Standard 12-lead resting ECG of each participant was obtained according to standard protocol After a period of rest of 5 minutes, the chest and extremities of each participant were exposed for placement of limb and chest electrodes. The ECG printouts were analyzed. Descriptive variables were expressed as means $\pm$ standard deviation. The lower and upper limits were determined using the 5th and 95th percentiles respectively. The comparison of the mean values of the male and female variables was done using the Student t-test. A p-value less than 0.05 was considered statistically significant. The results showed that the mean heart rate, QT interval, QTc interval, QRS duration, PR interval were 70.56 ± 10.99 bpm, 0.36 ± 0.03 s, 0.38 ± 0.03 s, 0.07 ± 0.01 s and 0.16 ± 0.02 s, respectively. Among young adults of Yoruba ethnicity, sex differences exist concerning heart rate, QTc, and QRS duration and these should be considered in ECG interpretation.

Keywords: Electrocardiogram; normal limits; mean values; sex differences; Young adults; Yoruba ethnicity.

*Authors for correspondence: diekoawoyinka@gmail.com, Tel: +234-8104959238

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INTRODUCTION

An electrocardiogram (ECG) is a useful screening and diagnostic non-invasive cardiac test. It has been reported that racial and sex differences exist with regards to ECG variables (Santhanakrishnan *et al.*, 2016; Macfarlane, 2018). Ethnic group-specific mean values and reference limits in the area of ECG are not available in Nigeria. Among the population of the Yoruba ethnic group in the South-West of Nigeria, baseline data for ECG assessment are not available. The aims of the study were to define the mean values, normal limits, and sex differences of heart rate, QT interval, QTc, PR interval, and QRS duration among young adults of Yoruba ethnicity.

The heart rate is the frequency of the heartbeat. QT interval is the total duration for ventricular depolarization and repolarization. It is measured from the onset of QRS complex to the end of T wave. QT interval is corrected for the heart rate and the derived variable is referred to as corrected QT interval (QTc). The PR interval is a measure of duration for atrioventricular conduction. It is measured from the onset of the P wave to the onset of the QRS complex. Changes in the PR interval may reflect physiological delay or signify abnormality in atrioventricular conduction. PR interval abnormality may be classified as prolongation (PR > 0.2 s) or shortening

(PR < 0.12s) (Holmqvist *et al.*, 2015). QRS duration is a measure of ventricular depolarization time and it has been shown to be a prognostic marker of coronary artery disease (Chen *et al.*, 2021).

MATERIALS AND METHODS

Study location: The study was carried out at the Obafemi Awolowo University Health Centre and the Department of Physiological Sciences, Obafemi Awolowo University, Ile-Ife.

Study design and population: It was a cross-sectional descriptive study. One thousand participants were grouped into two (500 males and 500 females). Inclusion criteria include; apparently healthy young adults between the ages of 18 and 40 years and Yoruba ethnicity. Exclusion criteria include the presence of systemic illness and other ethnic groups in Nigeria. The target population was the students of Obafemi Awolowo University community, Ile-Ife, Nigeria. The participants were educated about the procedure and informed consent was obtained from them.

Methodology: The standard 12-lead ECG of each participant was obtained in the supine position during quiet respiration using an electrocardiograph (Nihon Kohden

CardioFax). The chest and the limb extremities were exposed and 10 ECG electrodes were placed on the body surface of each participant according to internationally approved protocol. The chest lead recordings (V1 –V6) were obtained by the attachment of 6 electrodes to the anterior chest wall; V1 was placed at 4th intercostal space, right sternal edge, V2 was placed at 4th intercostal space left sternal edge, V4 was placed at the left 5th intercostal space along the mid clavicular line, V3 was placed at the midpoint between V2 and V4, V5 was placed at left 5th intercostal space along the anterior axillary line while V6 was placed at the left 5th intercostal space along the mid axillary line. The six limb leads (I, II, III, aVF, aVL, and aVR) were recorded with the aid of four electrodes attached to the distal end of the limbs (one electrode per limb). The PR interval, QT interval, RR interval, and QRS duration of each participant were measured from the ECG. The QT interval was measured using the tangent method and QT corrected (QTc) was calculated using Bazett's formula, $QTc = QT \text{ measured (ms)} / \sqrt{\text{preceding R-R interval}}$ (Dahlberg *et al.*, 2021). Heart rate was measured using the formula: Heart rate = 60/RR interval. All measurements were taken from lead II.

Data analysis: The data were analyzed using IBM SPSS version 20.0 software. The descriptive variables were expressed as means $\pm$ standard deviation. The lower and upper limits were determined using 5th and 95th percentile respectively. A comparison of the means of the male and female variables was done using the Student t-test. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of one thousand students (500 males and 500 females) between the ages of 18 and 40 years old, participated in this study. The mean age, heart rate, QT interval, QTc, QRS duration and PR interval for all participants were 21.06 ± 2.82 years, 70.56 ± 10.99 bpm,

0.36 ± 0.03 s, 0.38 ± 0.03 s, 0.07 ± 0.01 s and 0.16 ± 0.02 s respectively (Table 1).

Table 1:

Mean values and normal limit of selected ECG variables for all participants

ECG Variables	Mean $\pm$ SD N=1000	Normal limits (5th-95th percentile)
Heart rate (bpm)	70.56 ± 10.99	55.00-91.00
QT interval (s)	0.36 ± 0.03	0.32-0.40
QTc (s)	0.38 ± 0.03	0.34-0.44
QRS duration (s)	0.07 ± 0.01	0.06-0.10
PR interval (s)	0.16 ± 0.02	0.12-0.20

Normal limits at 5th and 95th percentile

There was a statistically significant difference in the heart rate of males and females with the males having a lower mean heart rate than females ($t = -12.994$, $p = 0.001$). The mean QT interval for females (0.35 ± 0.03 s) was lower than that for males (0.36 ± 0.03 s) but it was not significant ($p = 0.05$). The mean QTc interval was significantly higher ($p = 0.001$) in females (0.39 ± 0.03 s) than in males (0.37 ± 0.02 s). The mean QRS duration in males (0.08 ± 0.01 s) was significantly higher ($p = 0.001$) than that of the females (0.07 ± 0.01 s). The mean PR interval for males and females were the same (0.16 ± 0.02 s). The normal limits of ECG variables for all participants and each of the subgroups are illustrated in Tables 1-3.

DISCUSSION

The lower and the upper limits of the normal limits (55-91 bpm) of the heart rate from this study was lower than the lower and upper limits of the normal limits (61-93 bpm) reported among young adults in Nigeria by Ayoka *et al.*, (2014). This might be because the sample size (324) of the previous study was smaller than the one for this present study (1000).

Table 2:

Sex differences in selected ECG variables

ECG Variables	Male (n=500) Mean $\pm$ SD	Female (n=500) Mean $\pm$ SD	t- value	p-value
Heart rate (bpm)	66.38 ± 9.19	74.73 ± 11.06	- 12.994	0.001*
QT interval (s)	0.36 ± 0.03	0.35 ± 0.03	1.962	0.050
QTc (s)	0.37 ± 0.02	0.39 ± 0.03	- 11.664	0.001*
QRS duration (s)	0.08 ± 0.01	0.07 ± 0.01	7.419	0.001*
PR interval (s)	0.16 ± 0.02	0.16 ± 0.02	-0.497	0.620

*: significance at $p < 0.05$

Table 3:

Mean values and normal limits of selected ECG variables for males and females

ECG variables	Male Mean $\pm$ SD	n=500 Normal limits	Female Mean $\pm$ SD	n=500 Normal limits
Heart rate (bpm)	66.38 ± 9.19	52.00-84.00	74.73 ± 11.06	59.00-95.00
QT interval (s)	0.36 ± 0.03	0.32-0.40	0.35 ± 0.03	0.32-0.40
QTc (s)	0.37 ± 0.02	0.33-0.41	0.39 ± 0.03	0.34-0.45
QRS duration (s)	0.08 ± 0.01	0.06-0.10	0.07 ± 0.01	0.04-0.08
PR interval	0.16 ± 0.02	0.12-0.20	0.16 ± 0.02	0.12-0.20

Normal limits at 5th and 95th percentile

Moreover, while this present study was carried out among young adults of Yoruba ethnic group, the previous study was

not ethnic group specific. Furthermore, Chime *et al.*, (2020) reported a higher mean heart rate (73.46 ± 10.70 bpm) from

their study on healthy Nigerians in the Eastern part of the country. This disparity might be due to the fact that their study was on a wider age range (20-54 years). In contrast, Soliman and Rautaharju (2012) reported a lower mean heart rate of 68 ± 11 bpm among the United States White and Black adult population. This could be because they studied an older population (40 years and above), not of Yoruba ethnicity. The variation might be due to difference in environment and ethnicity.

From this present study the females had a significantly higher mean heart rate than males. This is in line with other reports (Ogunlade *et al.*, 2012; Ogedengbe *et al.*, 2023). The lower heart rate in males could be because of their higher vagal tone. Results from this present study could imply that males may be more prone to developing bradyarrhythmia and females may be more prone to developing tachyarrhythmia. The normal limits for QT interval from this study was similar to those reported by Ogunlade *et al.*, (2012) and Ayoka *et al.*, (2014) among the young adults in the Southwestern part of Nigeria. This could be because the participants of these other studies were predominantly of Yoruba ethnicity. The mean QT interval observed from this study was lower than the mean QT value of 0.41 ± 0.03 seconds observed by Soliman and Rautaharju (2012) in a population of black and white Americans. This could be because the other study was carried out on an older population (40 years and above). This is in concordance with a report by Dewhurst *et al.*, (2014) that QT interval increases with increasing age. There was no significant difference between the QT interval of males and females in this study. The mean QTc interval value (0.38 ± 0.03 s) obtained from this study was lower than the mean QTc of 0.44 ± 0.05 s reported by Tan *et al.*, (2016) among the Southeast Asian population. This might be because the study was among the Southeast Asian population of older age group (mean age of 65.7 years). Females had significantly higher QTc compared with their male counterparts. Several other studies have given similar reports (Funada *et al.*, 2008; Tan *et al.*, 2016; Ogedengbe *et al.*, 2023). It has been proposed that the gender related difference in QTc-interval is most likely the result of changes in sex-specific hormones (Vink *et al.*, 2018).

The normal limit for PR interval in this study was the same as the normal limit of PR interval (0.12 to 0.20 sec) obtained from a study carried out by Edemeka (2004) on adult (15 – 49 years) Nigerians resident in Lagos. The mean PR interval for males and females in this study was the same. On the contrary Tan *et al.*, (2016) reported that males had longer PR interval than females among the Asian population. This could be because the study was carried out on an older population with a mean age of 67.7 years.

With regards to QRS duration, the males showed a significantly higher QRS duration than the females in this study ($p < 0.001$). This is similar to the observations by Ogunlade *et al.*, (2012) and Tan *et al.*, (2016) among the Nigerian and Asian populations respectively. This difference might be due to the greater ventricular muscle mass in males than in females causing depolarization to take longer in males. This could mean that males might be more prone to the development of conduction abnormalities such as left or right bundle branch block. On the contrary Ogedengbe *et al.*, (2023) found no significant difference in QRS duration from their study on Young Africans.

In conclusion, the higher rate of cardiovascular disease incidence in recent times calls for greater specificity in defining normative values for cardiovascular variables. This study has been able to establish the mean values, normal limits of heart rate, QT interval, QTc, QRS duration and PR interval among young adults of Yoruba ethnicity. Furthermore, among young adults of Yoruba ethnicity, sex differences exist with regard to heart rate, QTc and QRS duration and these should be taken into consideration in ECG interpretation.

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REFERENCES

- Ayoka, A. O., Ogunlade, O., Akintomide, A. O., Akomolafe, R. O. and Ajayi, O. E. (2014). Normal limits of Electrocardiogram and cut-off values for left ventricular hypertrophy in young adult Nigerians. *Niger. J. Physiol. Sci.* 29: 063-066.
- Chen, X., Hansson, P. O., Thunström, E., Mandalenakis, Z., Caidahl, K. and Fu, M. (2021). Incremental changes in QRS duration as predictor for cardiovascular disease: a 21-year follow-up of a randomly selected general population. *Sci. Rep.* 11:13652. <https://doi.org/10.1038/s41598-021-93024-y>
- Chime P. E., Okenwa, W. O. and Oti, B. (2020). The Relationship Between Resting Heart Rate and Age in Adult Nigerians. *Am. J. Intern. Med.* 8(4):172-176. Doi: 10.11648/j.ajim.20200804.15
- Dahlberg, P., Diamant, U., Gilljam, T., Rydberg, A. and Bergfeldt, L. (2021). QT Correction using Bazett's formula remains preferable in long QT syndrome type 1 and 2. *Ann Noninvasive Electrocardiol.* 26 (1). <https://doi.org/10.1111/anec.12804>
- Dewhurst, M. J., Di Marco, L. Y., Dewhurst, F., Adams, P. C., Murray, A., Orega, G. P. *et al.*, (2014). Electrocardiographic reference values for a population of older adults in sub-Saharan Africa. *Ann Noninvasive Electrocardiol.* 19:34-42. <http://doi.org/10.1111/anec.12078>
- Edemeka, D. B. U. (2004). The electrocardiogram in healthy Nigerians resident in Lagos state. *Global Jnl of Pure and Applied Sciences* Vol.10(4) 567-570. <https://doi.org/10.4314/gjpas.v10i4.16440>
- Funada, A., Hayashi, K., Ino, H., Fujino, N., Uchiyama, K., Sakata, K. *et al.*, (2008). Assessment of QT intervals and prevalence of short QT syndrome in Japan. *Clin Cardiol.* 31(6):270-4. <https://doi.org/10.1002%2Fcl.20208>
- Holmqvist, F., Thomas, K. L., Broderick, S., Ersbøll, M., Singh, D., Chiswell, K., *et al.*, (2015). Clinical outcome as a function of the PR-interval—there is virtue in moderation: data from the Duke Databank for Cardiovascular Disease. *Europace.* 17:978-85. <https://doi.org/10.1093/europace/euu211>
- Macfarlane, P. W. (2018). The Influence of Age and Sex on the Electrocardiogram. *Adv Exp Med Biol.* 1065:93-106. https://doi.org/10.1007/978-3-319-77932-4_6
- Ogedengbe, J. O., Onaadebo, O., Okwuogori, J. K., Chori, B., Bara, A. D., Woyike, H. *et al.*, (2023). Normal values of electrocardiogram among healthy young adult African population. *Nigerian Journal of Cardiology* 20(1): 9-16.

- Ogunlade, O., Ayoka, A. O., Akintomide, A. O., Ajayi, O. E., Ukponmwan, O. E. and Ogunlade, O. B. (2012). Gender differences in electrocardiogram of young adults in south-western Nigeria. *Acta cardiologica*. 67(6):665-73.
- Santhanakrishnan, R., Wang, N., Larson, M. G., Magnani, J. W., Vasan, R. S., Wang, T. J., *et al.*, (2016). Racial Differences in Electrocardiographic Characteristics and Prognostic Significance in Whites Versus Asians. *J Am Heart Assoc*. 25;5(3):e0e002956 <https://doi.org/10.1161/JAHA.115.002956>. Erratum in: *J Am Heart Assoc*. 5(7). pii: e002058. <https://doi.org/10.1161/JAHA.116.002058>
- Soliman, E.Z. and Rautaharju, P.M. (2012). Heart rate adjustment of PR interval in middle-aged and older adults. *J Electrocardiol*. 45(1):66-9.
- Tan, S. J. E., Yap, J. F. C., Xu, C. F., Feng, L., Nyunt, S. Z., Santhanakrishnan, R. *et al.*, (2016). Association Of Age, Sex, Body Size And Ethnicity With Electrocardiographic Values In Community-based Older Asian Adults. *Heart, Lung and Circulation*. 25:705–7711. <https://doi.org/10.1016/j.hlc.2016.01.015>
- Vandenberk, B., Vandael, E., Robyns, T., Vandenberghe, J., Garweg, C., Foulon, V. *et al.*, (2016). Which QT Correction Formulae to Use for QT Monitoring? *J Am Heart Assoc*. 5(6). <https://doi.org/10.1161/JAHA.116.003264>
- Vink, A. S., Clur, S. B., Wilde, A. A. M. and Blom, N. A. (2018). Effect of age and gender on the QTc-interval in healthy individuals and patients with long-QT syndrome. *Trends Cardiovasc Med*. 28(1):64-75. <https://doi.org/10.1016/j.tcm.2017.07.012>.

Full length Research Article

The Effect of Vitamin D Level on Blood Glucose, HbA1c and Some Inflammatory Markers in Sudanese with Type 2 Diabetes Mellitus

Ali N.¹, Alaagib N.², Abdelatif A.³, Ali O.³, Elmagbol W.³, Alhojaily S.⁴,
Albokhadaim I.⁴, Shousha S.⁴, Alameen A.⁴

¹Pediatric Neurology Unit, Pediatric Department, Al - Sabah Hospital, Kuwait

²Sulaiman Alrajhi University, Al Qassim- Al Bukayriyah, , PO Box 777, Saudi Arabia

³Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum. Khartoum North, Sudan

⁴Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Saudi Arabia,

Summary: Diabetes Mellitus are more likely to occur in people with low vitamin D levels. In this study, Sudanese individuals diagnosed with type 2 diabetes mellitus (T2DM) had their serum glucose, HbA1c, vitamin D, and inflammatory markers assessed. In this case-control study, 120 individuals aged 35 to 69 were divided into two groups. The first group, 30 men and 30 women, had been diagnosed with Type 2 diabetes mellitus for a minimum of two years. The second group, the control group, comprised sixty healthy adults, evenly split between 30 males and 30 women. Every participant had a thorough medical history taken, with particular attention to the length of their diabetes, its medical history, and any previous problems. During the clinical examination and laboratory tests, the following parameters were evaluated: total WBC (1,000/ml), ESR (mm/hour), CRP (mg/L), HbA1c (%), and serum vitamin D3 (ng/ml). When 25(OH) vitamin D3 levels were utilized to assess T2DM patients, they showed significantly lower mean blood concentrations than controls: 23 (38.3%) had vitamin D insufficiency, 23 (38.3%) had vitamin D deficiency, and 14 (23.3%) had sufficient vitamin D. Vitamin D-deficient patients had significant increases in HbA1c, glucose, CRP, and total WBC. Additionally, when comparing the mean ESR values of diabetes patients to those of the control group, there was a statistically significant rise. ESR did not significantly alter depending on the controlling level. Males also had a numerically higher level of vitamin D3 than females. Compared to healthy normal controls, individuals with type 2 diabetes have noticeably reduced vitamin D3 levels. Furthermore, in T2DM patients, there was an association between elevated inflammatory markers and HbA1c and insufficiency in vitamin D.

Keywords: Vitamin D, blood glucose, HbA1c, inflammatory markers, Type 2 diabetes mellitus.

*Authors for correspondence: aomer@kfu.edu.sa, Tel: +966549373708

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INTRODUCTION

Over 80% of cases of diabetes mellitus are type 2 (Ostenson, 2001). It is a heterogeneous condition with a gradual onset that arises from the combination of environmental factors and polygenetic inheritance. The majority of T2DM patients are more susceptible to conditions like atherosclerosis, ischemic heart disease, ischemia, gangrene of the foot, and microangiopathy; diabetic nephropathies, including renal failure and glomerular damage; changes in the eyes, such as diabetic retinopathy and cataract; and neurological abnormalities (Schneider 2009).

Globally, vitamin D insufficiency is prevalent (Abuannai and O'Keefe, 2011). Previous study indicated that Vitamin D deficiency is associated with a higher chance of complications from diabetes, including cardiovascular disease (CVD) and renal impairment Al-Timimi and Ali (2013). Moreover, Lappe *et al.* (2007) reported that vitamin

D affects innate and adaptive immunity. High vitamin D concentrations may offer protection against inflammatory diseases, (Chu *et al.* , 2010; Elamin *et al.* , 2011). increased concentrations of inflammatory biomarkers, fibrinogen, WBC, or C-reactive protein (CRP). Additionally, vitamin D deficiency increases the risk of cardiometabolic disease and other chronic inflammatory diseases (Hewison 2012).

A combination of diabetes with vitamin D deficiency could result in more complications. Research on vitamin D supplementation for people with type 2 diabetes who are vitamin D deficient has produced conflicting results. Some have reported improved glucose control (Deluca 2004). Moreover, inadequate glycemic control was linked to vitamin D deficiency (Kaya *et al.* , 2018). Moreover, a few studies investigated the correlations between blood vitamin D levels and indicators of inflammation in the broader population (Jorgensen *et al.* , 2010; Ngo *et al.* , 2010).

According to the study, T2DM patients with vitamin D insufficiency had higher ESR than those with adequate vitamin D (Kaya *et al.* , 2018).

In addition to examining the vitamin D levels of the patients with type 2 diabetes, the study aimed to determine how low vitamin D levels related to glycaemic management, HbA1c, and specific inflammatory markers. Through the provision of vitamin D or close observation, the results of this study may improve the management of diabetic patients and avert major complications.

MATERIALS AND METHODS

This case-control study was carried out in the Khartoum state- Sudan. Samples were collected from clinics and hospitals in Khartoum state, such as Jabir Abu Alizz Diabetes Center, Soba University Hospital, and Turkish Teaching Hospital. The study included 60 T2DM patients aged 35–65 and 60 controls matched for age and gender (50% male and 50% female). Cases excluded from this study were pregnant females, renal, hepatic, parathyroid, smokers, alcoholics, cancer patients, and subjects on vitamin D supplementation, calcium, and drugs affecting vitamin D or calcium metabolism. A thorough medical history, including the length of the individual's diabetes, medical history, and history of complications from the disease, was taken from each participant. Accordingly, diabetic subjects were divided into good, moderate, and bad control subjects. Serum vitamin D3, glycated haemoglobin HbA1c, C-reactive protein (CRP), white blood cells (WBC), and erythrocyte sedimentation rate (ESR) were among the parameters examined in the clinical examination and laboratory tests. A trained nurse collected 5.8 ml venous blood samples from each enrolled subject under aseptic conditions. Each blood sample was divided into three parts: First part: 2 ml were collected in EDTA tubes to measure vitamin D, HbA1c, and TWC. Second part: 2 ml was collected in a lithium heparin tubes to measure fasting blood glucose and CRP. Third part: 1.8 ml was collected in an EDTA tubes to measure ESR. Serum glucose: Glucose concentration was determined by the enzymatic method using a kit (Randox Laboratories Ltd-London).

CRP: C-reactive protein was determined by the CRP-M100 as described by Zong *et al.* (2000).

TWBCs: Total white blood cells was performed in an improved Neubauer haemocytometer using Turk's solution

dilution fluid as described by Jain, (1993) by the Mindray BC-6800.

HbA1c: Glycated haemoglobin was determined by immunoassay using Cobas c501 as described by He *et al.* (2021)

Vitamin D: vitamin D was determined by electrochemiluminescence immunoassays using COBAS e411 as described by Yalla *et al.* (2019).

Erythrocyte Sedimentation Rate: The erythrocyte sedimentation rate test was determined by the Westergren method.

Statistical Analysis: Data were analyzed using Statistical Package for Social Science (SPSS). Analysis of variance Analysis of Variance (ANOVA) test (one way) and t-test was used to assess the significant difference among the groups. The differences are considered statistically significant at P value < 0.05. The results were presented as mean ± SD.

RESULTS

Glycemic profile and some inflammatory markers levels: Table (1) shows the two study groups' glycemic profile and some inflammatory markers levels. A significant (P<0.001) increase in the mean Glucose, HbA1c and ESR values in the diabetic patient compared to the control group's values. On the other hand, there was no discernible difference between the two groups' TWBC and CRP levels. When compared to controls, the serum level of vitamin D3 in diabetics was considerably (P<0.01) lower (ranging from 23.62±9.37 ng/ml to 34.85±32.34 ng/ml) Figure (1). Male respondents had significantly greater levels of vitamin D3 than female subjects did as well (Figure 2).

The influence of vitamin D levels on glycemic control and some inflammatory markers in patients with T2DM: The T2DM patients were evaluated based on their 25(OH) D levels; 23 (38.3%) patients had vitamin D deficiency, 23 (38.3%) patients had vitamin D insufficiency, and 14 (23.3%) patients were vitamin D-sufficient. Patients with vitamin D shortage had significantly higher levels of HbA1c, glucose, CRP, and TWB cells (P<0.01) compared to patients with vitamin D insufficiency and vitamin D-sufficient individuals. No significant difference in ESR was found (Table 2).

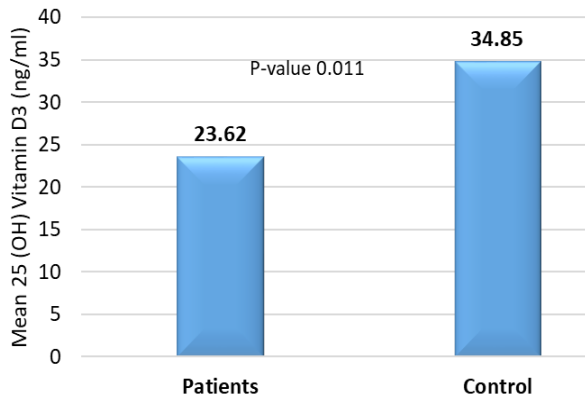
Table :1
Comparison of glycemic profile and some inflammatory markers.

Parameters	Diabetics (Mean±SD)	Control (Mean±SD)	P-value
Glucose (mg/dl)	141.0±56.74	86.28±11.23	0.000
CRP (mg/L)	11.14±12.10	12.72±9.08	0.418
TWC (1,000/ml)	7.25±4.32	6.67±2.44	0.368
ESR (mm/hour)	45.17±14.71	17.80±4.81	0.000
Vitamin D (ng/ml)	23.62±9.37	34.85±32.34	0.011
HbA1c (%)	8.90±2.09	-	-

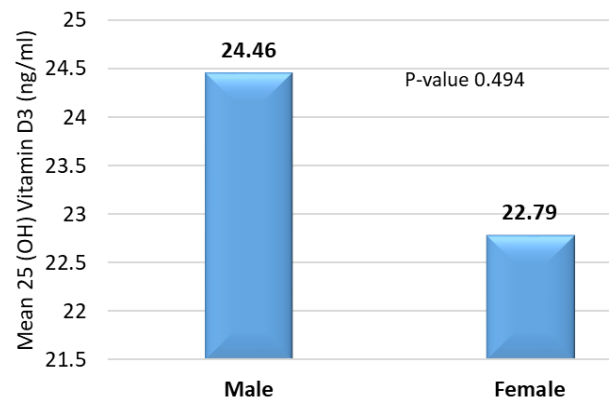
Table (2):

The influence of vitamin D levels on glycemic control and some inflammatory markers in patients with T2DM

Variables	Mean±SD			P-value
	Deficiency	insufficiency	sufficiency	
Sex M/F	12 (40)/11 (37)	12 (40)/11(37)	6 (20)/8 (27)	0.830
HbA1c (%)	10.4±1.84	8.42±1.80	7.15±0.84	0.000
Glucose (mg/do)	190.3±50.99	119.3±39.51	95.78±15.43	0.000
CRP (mg/l)	18.2±3.40	7.93±1.19	4.71±0.74	0.001
TWC (1,000/ml)	8.55±6.20	6.82±2.56	5.81±1.82	0.146
ESR (mm/hour)	43.4±15.57	46.9±12.95	45.07±16.64	0.737

**Figure 1:**

Serum level of 25 (OH) vitamin D3 in the diabetic and control group

**Figure 2:**

Relation between gender and Vitamin D3 level in participants (n = 60).

Correlation between vitamin D glycemic control and some inflammatory markers: Table 3 shows the relation between vitamin D and glucose levels, glycemic control, and other inflammatory markers. The following measures showed a strong connection with the vitamin D3 level: TWBC ($r = -0.308$, $p = 0.017$), HbA1C ($r = -0.605$, $p = 0.000$), CRP ($r = -0.500$, $p = 0.000$), and glucose ($r = -0.655$, $p = 0.000$). However, no correlation could be seen between ESR and vitamin D3 ($r = 0.100$, $p = 0.455$).

Influence of the diabetic's control on the blood glucose level, HbA1c and inflammatory markers: Table 4 shows that the diabetic subjects were divided into 3 subgroups according to glycemic control: 10 (16.7%) patients had good control, 15 (25.0 %) patients had moderate control, and 35 (58.3%) patients had inadequate control. HbA1c, Glucose, CRP, and TWB cells were considerably higher ($P < 0.001$) in patients with bad control than in moderate and good control; however, there was no significant difference in ESR regarding the controlling level.

Table (3):

Correlation of vitamin D with clinical and laboratory parameters in patients with T2DM.

Variables	R-value	P-value
Glucose (mg/dl)	-0.655**	0.000
HbA1c (%)	-0.605**	0.000
CRP (mg/l)	-0.500**	0.000
TWC (1,000/ml)	-0.308*	0.017
ESR (mm/hour)	0.100	0.455

DISCUSSION

The current finding indicated a significantly low serum level of vitamin D3 in diabetic patients compared to the control values. The vitamin D3 level directly influenced the glucose and HbA1c levels in the current result. This finding is expected so that vitamin D3 plays a role in body homeostasis. Also, vitamin D3 directly and indirectly influences the β -cell in the pancreas. Earlier studies have discovered a link between low levels of vitamin D and metabolic syndrome, a known risk factor for diabetes and other major causes of death in the US (Forrest and Stuhldreher, 2011; Binkley *et al.*, 2012; Fung *et al.*, 2012). It also plays a critical role in adult osteoporosis prevention. Further research has revealed that vitamin D involves more than maintaining calcium homeostasis.

Table (4):

Influence of the diabetic's control on the blood glucose level, HbA1c and inflammatory markers

Variables	Mean $\pm$ SD			P-value
	Good control	Moderate control	Bad control	
HbA1c (%)	6.66 ± 0.29	7.24 ± 0.26	10.3 ± 1.72	0.000
Glucose (mg/do)	98.6 ± 20.97	100.7 ± 21.06	170.4 ± 56.17	0.000
CRP (mg/l)	4.39 ± 2.12	5.31 ± 2.08	15.6 ± 2.41	0.002
TWC (1,000/ml)	5.58 ± 2.08	6.30 ± 2.16	8.13 ± 5.23	0.160
ESR (mm/hour)	48.10 ± 13.17	46.07 ± 14.68	43.94 ± 15.37	0.713

It is also vital for immune system function and cell proliferation (Christakos *et al.*, 2007), inhibits carcinogenesis, and delays the onset of other inflammatory diseases like T2D and cardiovascular disease (Roth 2007; Drake and Ng, 2010; Anagnostis *et al.*, 2010). Moreover, it has been revealed that vitamin D affects the development of insulin resistance, type 2 diabetes, and β -cell activity. Even though maintaining calcium homeostasis is the most well-known use of vitamin D, it is also believed to affect β -cell activity. This effect may be achieved by circulating 1, 25-dihydroxy vitamin D binding to the β -cell receptor (Peechakara and Pittas, 2008). As an alternative, 1-alpha-hydroxylase, expressed in β -cells, could activate 25(OH)D in response to vitamin D (Peechakara and Pittas, 2008). Vitamin D can also influence insulin secretion, a calcium-dependent function, by controlling extracellular calcium levels and calcium influx through β -cells (Sheshadri and Tamilselvan, 2011). The function of β -cell secretory may be adversely affected by any alterations in calcium flux (Pittas *et al.*, 2006; Palomer *et al.*, 2008).

The present study indicated that patients with vitamin D deficiency had significantly higher HbA1c and glucose levels compared to those with sufficient vitamin D and those with insufficient vitamin D. According to Badawi *et al.* (2014), vitamin D deficiency in type 2 diabetes may have an effect on beta cell function and the onset of type 1 diabetes. Vitamin D deficiency and HbA1C are significantly correlated, and there is an inverse association between vitamin D and insulin resistance, according to Yiu *et al.* (2011). Forouhi *et al.* (2014). These authors pointed out that when there was baseline vitamin D insufficiency, there was a ten-year risk of higher glucose concentrations while fasting, glucose concentrations throughout the two hours, and a metabolic syndrome risk factor score. Similar findings were reported by the majority of prospective studies on young people recently diagnosed with diabetes (Daa *et al.*, 2014).

The research findings indicate a statistically significant rise in the average ESR values among individuals with diabetes mellitus in contrast to the control group. On the other hand, Table 1 indicates that there was no remarkable difference in TWBC and CRP levels between the two groups. Compared to patients with vitamin D insufficiency and those with adequate vitamin D, those with vitamin D deficit had significantly higher CRP and TWBC levels. Compared to individuals with moderate and well-managed blood sugar, those with poorly controlled blood sugar had significantly higher levels of CRP and TWBC (Table 4). Nonetheless, there was no discernible variation in ESR concerning the controlling level. This research supports a prior meta-analysis of prospective studies that discovered a substantial correlation between CRP and the risk of type 2 diabetes (T2DM) (Wang *et al.*, 2013). Lymphocytes and insulin levels correlate positively (Ryder *et al.*, 2014). A comprehensive review and meta-analysis found a clear correlation between higher WBC counts and a higher risk of type 2 diabetes (Gkrania-Klotsas *et al.*, 2010). Further research yielded some results indicating that inflammation plays a role in the onset of diabetes. Diabetes mellitus and ESR did not significantly correlate, according to Schmidt *et al.* (1999).

Increased risk of cardiometabolic disease and other chronic inflammatory disorders has been associated with elevated levels of inflammatory biomarkers, such as C-reactive protein (CRP), white blood cell count (WBC), or plasma fibrinogen (Hewison 2012). High vitamin D concentrations may offer protection against inflammatory diseases, according to certain research (Chu *et al.*, 2010; Elamin *et al.*, 2011). The findings of this investigation contradict with those of a few other studies (Ostenson 2001; Ngo *et al.*, 2010) that looked at the relationships between inflammatory markers and blood vitamin D levels. The current study's findings conflict with another study that found T2DM patients with vitamin D insufficiency had higher ESRs than those with adequate vitamin D. ESR and vitamin D levels were inversely correlated (Kaya *et al.*, 2018).

Males had considerably greater levels of vitamin D3 than females in the current investigation. This could be attributed to the habits of dressing and less exposure to the sun for females in the Middle East and North Africa. Previous studies showed how clothing (long sleeves and pants), sunscreen (applied liberally and frequently), and behaviour (seeking shade) can reduce the amount of skin surface area that is exposed to solar UVB ray penetration and consequently limit the amount of vitamin D3 synthesis that occurs on the skin (Matsuoka *et al.*, 1987; Matsuoka *et al.*, 1992).

Furthermore, this study indicated that the poorly controlled blood sugar group had significantly higher HbA1c and glucose levels compared to the individuals with moderately and well-managed blood sugar, those with. Regarding diabetes self-management, it is crucial to have reliable and valid measures (McNabb 1997). Several self-care behaviors in individuals with diabetes are associated with positive outcomes. These encompass nutritious dietary choices, regular physical activity, blood sugar monitoring, adherence to prescribed medications, effective problem-solving abilities, healthy coping mechanisms, and behaviors aimed at reducing risk (Shrivastava *et al.*, 2013). These behaviors demonstrated a positive correlation with effective glycemic control, a decrease in complications, and an enhancement in quality of life (Deakin *et al.*, 2005).

In conclusion, the current investigation found that T2DM patients had considerably lower levels of 25(OH) vitamin D3 compared to healthy controls. The low levels of 25(OH) vitamin D3 elevated inflammatory markers. Vitamin D supplements may be helpful in the prevention and treatment of type 2 diabetes since vitamin D levels have been related to glycemic control in diabetics. Further research is needed to fully understand the benefits of including vitamin D supplements in the type 2 diabetes treatment plan.

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Author's Contribution: This work was carried out in collaboration among all authors. Authors NM, AOA and NAA designed the experiment. NM performed the experiment, AOA and NAA performed the statistical data analysis and wrote the first version of the manuscript. Authors OHA, WAE, SA, IA, and SSS revised this article to confirm scientific data authentication and

participated in preparing the manuscript. All authors revised and approved the final version of the manuscript.

Ethics approval:

Ethical approval was granted from Khartoum State- Ministry of Health- Research Department

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

REFERENCES

- Abuannai M, O'Keefe JH (2011). Vitamin D and cardiovascular health. *Primary Care Cardiovasc. J.* 4: 59–62.
- Al-Timimi DJ, Ali AF (2013). Serum 25(OH) D in Diabetes Mellitus Type 2: Relation to Glycaemic Control. *JCDR.* 7: 2686–2688.
- Anagnostis P, Athyros VG, Adamidou F, Florentin M, Karagiannis, A. (2010). Vitamin D and cardiovascular disease: A novel agent for reducing cardiovascular risk. *Curr. Vasc. Pharmacol.* 8: 720–30.
- Badawi A, Sayegh S, Sadoun E, Al-Thani M, Arora P, Haddad PS. (2014). Relationship between insulin resistance and plasma vitamin D in adults. *Diabetes Metab. Syndr. Obes.* 7: 297–303.
- Binkley N, Ramamurthy R, Krueger D. (2012). Low vitamin D status: Definition, prevalence, consequences, and correction. *Rheum. Dis. Clin. North. Am.* 38: 45–59.
- Boltri JM, Okosun IS, Davis-Smith M, Vogel R. (2005). Hemoglobin A1C in diagnosed and undiagnosed black, Hispanic, and white persons with diabetes: Results from NHANES 1999–2000. *Ethn. Dis.* 15: 562–67.
- Christakos S, Dhawan P, Benn B, Porta A, Hediger M, Oh GT, Joshi S. (2007). Vitamin D. *Ann. N. Y. Acad. Sci.* 1116: 340–348.
- Chu MP, Alagiakrishnan K, Sadowski C (2010). The cure of ageing: vitamin D – magic or myth?. *Postgrad. Med. J.* 86: 608–616.
- Daga RA, Laway BA, Shah ZA, Mir SA, Kotwal SK, Zargar AH. (2014). High prevalence of vitamin D deficiency among newly diagnosed youth-onset diabetes mellitus in North India. *Arq. Bras. Endocrinol. Metab.* 56: 423–27.
- Deakin TA, McShane CE, Cade JE, Williams R. (2005). Group based training for self-management strategies in people with type 2 diabetes mellitus. *Cochrane database of systematic reviews.* (2).
- Deluca HF. (2004). Overview of general physiological features and functions of vitamin D. *Am. J. Clin. Nutr.* 80: 1689S–1696S.
- Drake MT, Ng AC. (2010). Vitamin D deficiency in multiple myeloma. *J. Clin. Oncol.* 2: 1–4.
- Elamin MB, Abu Elnour NO, Elamin KB (2011). Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J. Clin. Endocrinol. Metab.* 96: 1931–1942.
- Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. (2014). Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance. *Diabetes* 57: 2619–25.
- Forrest KZ, Stuhldreher WL. (2011). Prevalence and correlates of Vitamin D deficiency in US adults. *Nutr. Res.* 31: 48–54.
- Fung GJ, Steffen LM, Zhou X, Harnack L, Tang W, Lutsey PL, Horn LV. (2012). Vitamin D intake is inversely related to risk of developing metabolic syndrome in African American and white men and women over 20 y: The Coronary Artery Risk Development in Young Adults study. *AJCN* 96: 24–29.
- Gkrania-Klotsas E, Ye Z, Cooper AJ, Sharp SJ, Luben R, Biggs ML, Landenberg C. (2010). Differential white blood cell count and type 2 diabetes: Systematic review and meta-analysis of cross-sectional and prospective studies. *PLoS One* 5: e13405. doi: 10.1371
- He D, Kuang W, Yang X, Xu M. (2021). Association of hemoglobin H (HbH) disease with hemoglobin A1c and glycated albumin in diabetic and non-diabetic patients. *Clinical Chemistry and Laboratory Medicine (CCLM).* 591127–32.
- Hewison M. (2012). Vitamin D and the immune system: new perspectives on an old theme. *Rheumc. Dis. Clin. North. Am.* 38: 125–139.
- Jain NC. (1993). *Essentials of Veterinary Haematology.* Lea and Febiger, Philadelphia., pp. 349–380.
- Jorgensen SP, Agnholt J, Glerup H. (2010). Clinical trial: vitamin D3 treatment in Crohn's disease – a randomized double-blind placebo-controlled study. *Aliment. Pharmacol. Ther.* 32: 377–383.
- Kaya T, Akcay EU, Erturk Z, Ergenc H and Tamer A. (2018). The relationship between vitamin D deficiency and erythrocyte sedimentation rate in patients with diabetes. *Turk. J. Med. Sci.* 48: 424–429.
- Lappe JM, Travers-Gustafson D, Davies KM (2007). Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am. J. Clin. Nutr.* 85: 1586–1591.
- Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. (2002). Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 30: 771–77.
- McNabb WL. (1997). Adherence in diabetes: can we define it and can we measure it?. *Diabetes care.* 1:215.
- Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick, MF. (1987). Sunscreens suppress cutaneous vitamin D3 synthesis. *J. Clin. Endocrinol. Metab.* 64: 1165–68.
- Matsuoka LY, Wortsman J, Dannenberg MJ, Hollis BW, Lu Z, Holick MF. (1992). Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3. *J. Clin. Endocrinol. Metab.* 75: 1099–1103.
- Ngo DT, Sverdlov AL, McNeil J. (2010). Does vitamin D modulate asymmetric dimethyl arginine and C-reactive protein concentrations?. *Am. J. Med.* 123: 335–341.
- Ostenson CG. (2001). The pathophysiology of type II diabetes mellitus: An over view. *Acta Physiol. Scand.* 171: 241–247.
- Palomer X, Gonzales-Clemente JM, Blanco-Vaca F, Mauricio D. (2008). Role of Vitamin D in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obes. Metab.* 10: 185–97.
- Peechakara SV, Pittas AG. (2008). Vitamin D as a potential modifier of diabetes risk. *Nature clin. pract. endocrinol. Metab.* 4: 182–83.
- Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB. (2006). Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 29: 650–56.

- Roth DE. (2007). Bones and Beyond: An update on the role of Vitamin D in child and adolescent health in Canada. *Appl. Physiol. Nutr. Metab.* 32: 770-777.
- Ryder E, Diez-Ewald M, Mosquera J, Fernández E, Pedreañez A, Vargas R, Fernandez N. (2014). Association of obesity with leukocyte count in obese individuals without metabolic syndrome. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews* 8: 197-204.
- Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. (1999). Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 353: 1649-52.
- Schneider DJ. (2009). Factors Contributing to Increased Platelet Reactivity in People with Diabetes. *Diabetes care* 32: 525-527.
- Sheshadri KG, Tamilselvan B. (2011). Role of Vitamin D in Diabetes. *J. Endocrinol.* 1: 47-56.
- Shrivastava SR, Shrivastava PS, Ramasamy J. (2013). Role of self-care in management of diabetes mellitus. *Journal of diabetes & Metabolic disorders.* 12:1-5.
- Takiishi T, Gyseman C, Bouillon R, Mathieu C. (2010). Vitamin D and diabetes. *Endocrinol. Metab. Clin. N. Am.* 39: 419-46.
- Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Liu LG. (2013). Inflammatory markers and risk of type 2 diabetes: A systematic review and meta-analysis. *Diabetes Care* 36: 166-75.
- Yalla N, Bobba G, Guo G, Stankiewicz A, Ostlund R. (2019). Parathyroid hormone reference ranges in healthy individuals classified by vitamin D status. *Journal of endocrinological investigation.* 42:1353-60.
- Yiu YF, Chan YH, Yiu KH, Siu CW, Li SW, Wong LY, Lee SWL, Tse HF. (2011). Vitamin D deficiency is associated with depletion of circulating endothelial cells and endothelial dysfunction in patients with type 2 diabetes. *J. Clin. Endocrinol. Met.* 96: E830-E35.
- Zong Z, Kan L, Luo Y, Wang C, Wang X, Wang M, Xiong D, Zhang X. (2020). Performance evaluation of the XPEN60 CRP&SAA, a novel automated hematology analyzer, in detecting complete blood count, C-reactive protein, and serum amyloid A. *Journal of clinical laboratory analysis.* 34:e23395.

Full length Research Article

Exercise Attenuates the Effect of High Salt Intake on the Cardiovascular Function, Oxygen Saturation, Lung Function and Renal Function of Young Men

***Jaja S.I. and Adekogbe O.A.**

Department of Physiology, Faculty of Basic Medical Science College of Medicine university of Lagos.

Summary: Coronary heart disease (CAD), respiratory disease, and early-onset renal failure, which until recently were only common in high-income countries, are now the dominant source of morbidity and mortality among young Nigerian adults. However, epidemiological studies have suggested the possibility of high dietary salt intake and physical inactivity as behavioural factors that may be responsible for this growing trend. Therefore, the purpose of this study was to determine the influence of elevated salt intake and physical activity and inactivity on the pulmonary function, cardiovascular, and renal function of young Nigerian men. **Methods and materials:** A total of 20 subjects, comprising 10 non-exercising young men (control) and 10 exercising young men, participated in the study after obtaining an approval from the ethical committee of the animal and human research bioethics department. Lung function, oxygen saturation, blood pressure, urine volume, urine pH, and urine Na⁺ and K⁺ concentration were measured under resting conditions before and after five days of 200mmol of salt loads in both groups. The data was analyzed using the SPSS Statistics software package Version 19. The unpaired t-test was used to calculate the P-value across the groups. The paired t-test was used to calculate the p-value within the groups. Statistical significance was reached when $P < 0.05$. **Results:** Salt loading had no significant effect on the pulmonary function of the control subjects. However, salt loading worsened the pulmonary function values of the exercising subjects, with FEV1, FVC, and PEFR decreased significantly by -0.05 ± 0.05 L, -0.003 ± 0.01 L, and -20.20 ± 7.11 L/min, respectively, without affecting oxygen saturation (SPO2) and FEV1%. Salt loading caused a greater increase in the blood pressure parameters of the non-exercising subjects, with systolic pressure, diastolic pressure, mean arterial pressure, and pulse pressure significantly increased by 18.00 ± 2.04 mmHg, 11.90 ± 1.52 mmHg, 13.97 ± 1.98 mmHg, and 6.20 ± 0.24 mmHg, respectively. In summary, exercising subjects eliminate salt loads more effectively than the non-exercising subjects to reduce salt retention. This might be as a result of the trigger of some pathophysiological mechanisms that alters vital bodily functions such as respiratory function, renal function, and cardiovascular functions.

Keywords: Oxygen Saturation, pro-inflammatory cytokines, Hyperinsulinemia, angiotensin-2, and Nitric oxide.

*Author for correspondence: Email: sjaja@unilag.edu.ng, Tel: +234-80367291922

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INTRODUCTION

Exercise is a stressful condition that serves as a means of testing the physical capabilities and physiological responses of an individual. It forms the basis for good health and wellbeing with the ability or capacity to endure stress. The burden of unhealthy diets constitutes a major public health concern globally and urgent action is needed to address this growing trends. The over-production and over-consumption of foods and beverages that do not meet healthy nutritional standards, mainly manufactured foods are of great concern due to the high amount of sodium they contain. In most developed and developing countries, a significant proportion of sodium in the diet comes from processed foods, particularly from seasoning and condiments used for cooking to add taste to food or even from fast foods we get from restaurants and canteens. The global mean intake of sodium is about 4310 mg/day (equivalent to 10.78 g/day of

salt) which is more than double the World Health Organization's recommendation for salt intake in adults (< 2000 mg of sodium per day, an equivalent to < 5 g of salt per day). This doubling in the consumption of salt in this time and age of modern civilization has really become a public health concern. An estimated 1.89 million deaths each year have been associated with consuming too much sodium, a well-established cause of raised blood pressure and an increased risk of cardiovascular disease (IHME, 2019). Scientists have become aware of the health risks associated with high salt intake, which include hypertension, chronic obstructive pulmonary conditions, renal failures, and other anomalies triggered by high dietary salt intake (AHA, 2009). The ability of regular exercise to augment the bioavailability of Nitric Oxide can have a beneficial influence to counter the effect of this high dietary salt intake and its associated health risk on the respiratory system (Uematsu *et al.*, 1995). Several epidemiological

studies have linked dietary salt to the prevalence and severity of obstructive lung conditions particularly in a population on a high salt diet (Burney, 1995). Recent studies show dietary salt loading increases serum osmolality by stimulating the systemic increase in pro-inflammatory cytokines (Philipp *et al.*, 2006), which has been found to have the potential to damage the lungs and reduce lung function. Another fact points out the influence of dietary salt on circulating blood volume and consequently on hemodynamic functions as another possible link that may relate the effect of dietary salt on lung function and oxygen saturation. The aim of the study is to evaluate the effect of dietary factors such as high salt intake and lifestyle choices of active or sedentary lifestyle on lung function, oxygen saturation, blood pressure, and renal function amongst young men.

MATERIALS AND METHODS

Ethical consideration: An ethical approval with CMUL/ACUREC/05/2012/1635 was obtained from the research and bioethics committee of the animal and research bioethics department before the experiment. An informed consent form was also issued and completed by each participant before the commencement of the experiment.

Participants: The experiment was carried out on 20 healthy young male subjects. The subjects were all on a normal diet and none of them were on any type of medication for any disease condition that could affect the result obtained from the experiments. Selection of subjects was based on the selection criteria below. The Selection of the participants was done based on the following criteria: No clinical history of asthma; No clinical history of sickle cells anemia, No history of alcohol or substance abuse. Only healthy male volunteers between the age of 20 – 27 years were eligible for the study.

Research Design For the purpose of the study, the participants were all students of the College of Medicine, University of Lagos (CMUL), Idi-araba, Lagos, Nigeria; all living under hygienic condition within the premises of the medical student hostel, CMUL, Idi-araba. A food intake survey was designed to identify sources of dietary salt. Questionnaires were administered to all participants to assess different patterns of meal consumption. The questionnaire was divided into five sections which includes frequency of meal consumption on campus, frequency of meal consumption off campus, frequency of consuming certain food groups that may be a potential source contributing to dietary salt, frequency of consuming certain local dishes that are high in salt, frequency of preparing their own meal. The food group listed in the food intake survey were selected from nutrient composition of Nigerian foods (Tindel, 1985) based on food identified by (Towobola *et al.*, 2013) as being high in salt and food group with at least moderate salt content (>100mg sodium /100g). The food intake survey questionnaire (appendices-1) which was administered to all volunteers was aimed at establishing the dietary intake of all participants. The result of the food survey was used in grouping the participants

Grouping of participants: The participants were grouped into two groups of ten people each. The groups are; the exercise group, consisting of undergraduate medical students who engaged more frequently in exercises and consumed more frequently food high in salt according to the result of the food intake survey. The second group is the non-exercising group that consisted of participants from among the undergraduate medical students of the university who do not engage more frequently in exercise and consumed food high in salt according to the food intake survey. Participants in each group served as their own control. Anthropometric parameters such as height (metres), weight (Kg), Body Mass (Kg/m²) index, Body Surface area (m²) were measured for each participants. Participants in each group were subjected to a baseline measurement of lung function, oxygen saturation and blood pressure before the consumption of a high salt diet of 200mmol sodium for five days to increase their salt intake. Exercise group engage in an endurance exercise of cycling on a bicycle ergometer to measure the same parameters.

Salt-loading: Subjects were loaded with 200mmol sodium chloride solution on a daily basis for a period of 5 days (Tzemoz *et al.*, 2008). This was prepared by dissolving 10.9g scientific graded salt (sigma - Aldrich, AHANALA GRADE) in 700mls of water mixed with 300mls of orange squash to make up a whole salt solution. The treatment was well tolerated by all subjects.

Measurement of lung function (FEV1, FVC, FEV1, %) and oxygen saturation (SPO2): The lung function and oxygen saturation were measured using the Spirolab III, an open circuit spirometer connected to a pulse oximeter (MIR model). A multi-tasking and a versatile spirometer with a high-performance personal computer software (winspiro - Pro) that automatically generated a participant data card with a preview of the spirometry curve. The spirolab 111 measures the lung function (FVC, FEV, FEV1%) by forcefully breathing in and forcefully breathing out into the turbine of the SPIROLAB via a disposable mouthpiece with the nose clipped. The oxygen saturation was measured by a pulse oximeter connected to the spirolab II1 via a cable. It measures the oxygen saturation by inserting the index finger into the probe of the pulse oximeter, until it picks up the signal which it sends to the spirolab III for processing before displaying on the screen. The lung function and oxygen saturation were measured in the two groups of participants before and after salt loading to have a baseline FVC, FEV1, FEV1% and SPO2 (PRE-TEST) and a test FVC, FVC1, FEV1% and SPO2 (post-test)

Collection of 12 hours urine sample: This was carried out before and after the salt loading protocol. The 12 hours urine sample was collected before and after salt loading into a 2 liters urine collection bottle containing chloroform. The chloroform was used as preservative to prevent bacterial growth in the urine. Subjects were given verbal instructions on how to collect a 12-hour urine sample. They were told to start collection at 9.00pm, urine voided before this time was discarded. Thereafter urine voided as from 9.00pm was collected into the 2 liters urine collection bottle containing chloroform for duration of 12hours i.e. 9.00am of the

following day. Using a 500mls measuring cylinder, the volume of the 12 hours urine sample was accurately measured. The pH of the urine samples were also determined using a pH meter.

Determination of urine sodium and potassium concentration: The concentration of sodium and potassium in 12 hours urine samples were analyzed using the ion selective electrode method (ISE method). This involves the use of ISE 600 Analyzer (ISE 600 by SFRI) which analyzes the urine samples after calibration by aspirating sample of urine to determine the concentration of Na⁺ and K⁺ in urine. Results are displayed on the screen after analysis. The observed values of the samples were expressed in mmol/L Na⁺ and K⁺

Determination of blood pressure (mmHg): The blood pressure for each participant was measured in the sitting position with their back rested firmly on the chair and their feet flat on the floor. Subjects were allowed a 5-minute period of rest in this position before commencement of blood pressure measurement.

Subjects were not allowed to wear constrictive clothing on the upper arm in order to avoid tourniquet effect above the Riva – roc-cuff. Subjects arm was supported at the level of the heart. The Riva -Rocci cuff of the sphygmomanometer was placed in a way to encircle at least 80% of the arm circumference. Arterial blood pressure measurement was taken from the brachial artery pulsation of the right arm with the arm in the supine position; systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded as the first and fifth korot- koff phases respectively (Kim *et al.*, 2023). The cuff was deflated at the rate of 2mmHg per sec. Measurements were taken to the nearest 2mmHg to avoid digit preference. Digit bias or digit prejudice is a situation in which the observer records an inappropriate excess of “Zero” as the last digit in blood pressure recording/ depending on the subjects’ circumstance; the observer records the blood pressure just below or just above a cutoff point (Wing field *et al.*, 2002). After initial measurement to avoid “White coat” effects, three readings were taken with one minute interval in between for the subjects to relax: the average of the three measurements was recorded.

Measurement of arterial blood pressure: Arterial blood pressure measurement was measured using a non- invasive indirect auscultatory method by using the stethoscope and a mercury Sphygmomanometer. The gold standard in the measurement of blood pressure is the use of mercury sphygmomanometer and the Korotkoff sound technique using the auscultatory method (Pickering *et al.*, 2005). The auscultatory method is the mainstay of blood pressure methods but is gradually being supplanted by other techniques due to increase in the use of automated blood pressure measuring devices (Pickering *et al.*, 2005). The mercury sphygmomanometer consists of a mercury column inside a vertical column to which is attached the riva – rocci inflatable Cuff and an inflator. This device has changed little since it was designed about six decades ago except for the fact that modern versions have very little risk of spilling mercury if dropped. The mercury sphygmomanometer is unique in its simplicity and there is negligible difference in

the accuracy of different brands of the instrument (Pickering *et al.*, 2005). Baseline blood pressure (mmHg) was determined following the standardized protocol developed by the international collaborative study of hypertension in blacks (ICSHIB) and the American Heart Association (AHA) recommendation for blood pressure measurement in Human (Pickering *et al.*, 2005).

Determination of Pulse pressure and Mean arterial pressure: Pulse pressure was determined by subtracting the diastolic blood pressure from the systolic blood pressure. Mean arterial blood pressure was determined by adding one third of pulse pressure to the diastolic pressure. $PP = \text{Diastolic Blood Pressure (DBP)} - \text{Systolic Blood Pressure (SBP)}$
 $MAP = 1/3 \text{ Pulse Pressure (PP)} + \text{Diastolic Blood Pressure (DBP)}$

Determination of change and % change of parameters after salt loading: Changes in the parameters are those changes computed by subtracting an initial value from a final value divided by an initial value. $\text{Change } (\Delta) = \text{final value} - \text{initial value}$

$\text{Initial value. \% change} = (\text{final value} - \text{initial value}) \times 100$
 Initial value

Data have been summarized and presented as mean $\pm$ standard error of mean (S.E.M) and range. A statistical test of significance was prepared at 95% confidence level in order to show how significant the results would deviate from control and thus able to draw conclusion. The student’s unpaired t-test was used to calculate the P-value across the group. The students paired t-test was used to calculate the pvalue within the group. Statistical significance was adopted when $P < 0.05$.

RESULTS

Results of food questionnaire: Most of the students had food on campus on a daily basis. Only few of the students had food off campus. Most students added soy, salt and sauce to served food on a daily basis. Most of the students consumed more of bakery, salted egg and instant meal than they did consume local foods. Most of the students hardly did their own cooking. Most of the student admitted of adding salt, sauce and fish sauce while cooking. The result of the questionnaire showed that most food consumed more frequently by the students are foods that fall under the category of foods that are high in salt contents as identified by Towobola *et al.* (2013).

Comparison of baseline anthropometric parameters in non- exercising and exercising young men: Comparison of baseline anthropometric parameters in both groups of subjects (Table 1) reveal they were within the same age, height, weight, body surface area and body mass index limit. There was no significant difference between the control group and the exercising group.

Comparison of baseline ventilatory function in non-exercising and exercising young men: Comparison of the baseline ventilatory function in both groups (Table 2) reveals that oxygen saturation (SPO₂), forced expiratory volume in 1 sec (FEV₁), forced vital capacity (FVC) and

peak expiratory flow rate (PEFR) differed significantly in both groups (Fig. 1-3) with $P < 0.05$ and 0.001 respectively. Percentage forced expiratory volume in 1 sec (FEV1%) in both groups were similar.

Comparison Of Baseline Blood Pressure in Non-Exercising And Exercising Young Men: Comparison of

Table 1:
 Baseline anthropometric parameters in non-exercising and exercising young men

Parameters	Non-Exercising Subjects (Mean $\pm$ S.E.M)	Range	Exercising Subjects (Mean $\pm$ S.E.M)	Range	Remarks
Age (yr)	23.00 $\pm$ 0.88	20-28	22.90 $\pm$ 0.91	20-27	NS
Height (m)	1.70 $\pm$ 0.02	1.60-1.80	1.75 $\pm$ 2.80	1.64-1.91	NS
Weight (kg)	62.00 $\pm$ 2.05	53-71	68.30 $\pm$ 2.77	53-85	NS
BMI (kg/m ²)	21.20 $\pm$ 0.46	18.83-23.16	22.39 $\pm$ 1.27	17.31-31.21	NS
BSA (M2)	1.72 $\pm$ 0.03	1.54-1.90	1.82 $\pm$ 0.04	1.64-1.96	NS

Values are expressed as mean $\pm$ S.E.M; NS =Not significant

Table 2:
 Comparison of baseline ventilatory function in non-exercising and exercising young men

N= 20	MEAN $\pm$ S.E.M	
PARAMETERS	Non-Exercising Subjects	Exercising Subjects
FVC (Litre)	2.73 $\pm$ 0.15	3.93 $\pm$ 0.20***
FEV1 (Litre)	2.45 $\pm$ 0.13	3.58 $\pm$ 0.21*
FEV1%	90.49 $\pm$ 2.47	90.94 $\pm$ 2.17
PEFR (L/MIN)	411.40 $\pm$ 22.70	511.30 $\pm$ 24.83***
SPO2 (%)	95.95 $\pm$ 0.55	97.40 $\pm$ 0.18*

Values are expressed as mean $\pm$ S.E.M; SIG =Significant .NS =Not significant

*= $P < 0.05$, ***= $P < 0.001$ respectively

FEV1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; PEFR = Peak expiratory flow rate; SPO2 = oxygen saturation

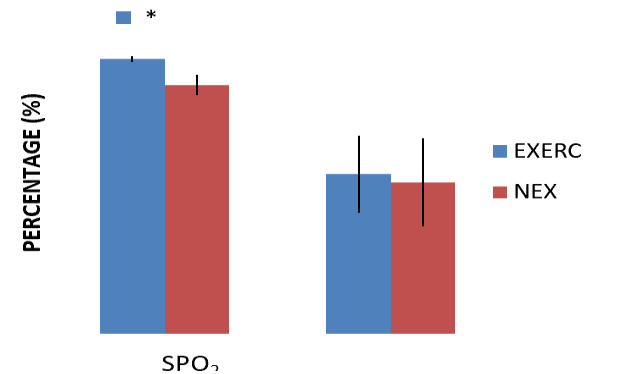


Figure 1:
 Comparison of oxygen saturation (SPO2) and FEV1% in non-exercising and exercising young men. * = $P < 0.05$

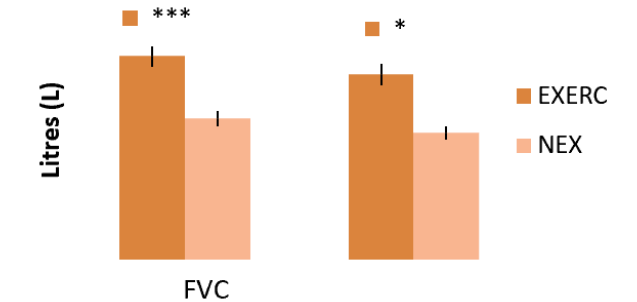


Figure 2:
 Comparison of forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV1) in non-exercising and exercising subjects. *** = $P < 0.001$ and * = $P < 0.05$

baseline blood pressure parameters in both groups (Table 3) revealed that only systolic blood pressure differed significantly between the groups. There was no significant difference in the diastolic blood pressure, mean arterial blood pressure and pulse pressure of both groups (Fig. 4).

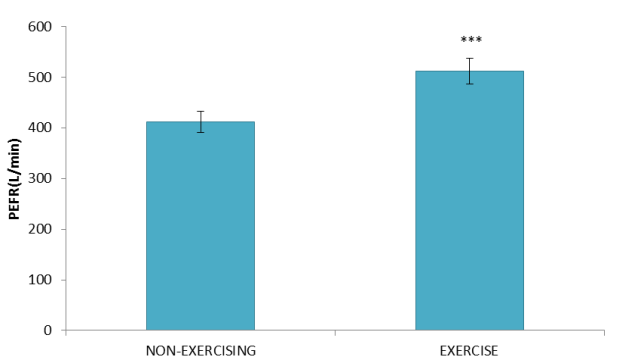


Figure 3:
 Comparison of peak expiratory flow rate (PEFR) in non-exercising and exercising subjects. *** = $P < 0.001$

Table 3:
 Comparison of baseline blood pressure in non-exercising and exercising young men

N= 20	Mean $\pm$ S.E.M		
Parameters	Non-Exercising Subjects	Exercising Subjects	Remark
SBP (mmHg)	107.20 $\pm$ 2.06	100.50 $\pm$ 1.53*	SIG
DBP (mmHg)	70.00 $\pm$ 2.84	63.50 $\pm$ 3.14	NS
PP (mmHg)	37.20 $\pm$ 3.58	37.00 $\pm$ 2.72	NS
MABP (mmHg)	82.40 $\pm$ 3.26	75.83 $\pm$ 2.01	NS

Values are expressed as mean $\pm$ S.E.M; SIG =Significant

NS =Not significant; *= $P < 0.05$

SBP = systolic blood pressure; DBP = diastolic blood pressure;

MABP = mean arterial blood pressure; PP = Pulse pressure

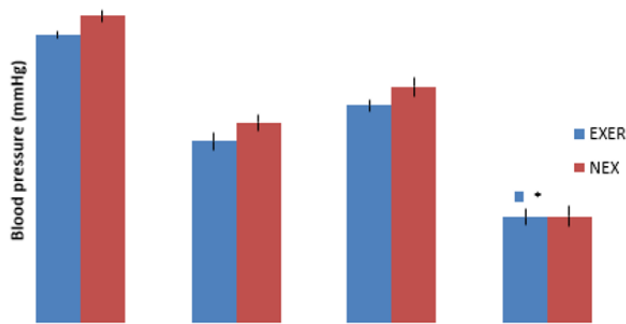


Figure 4: Comparison of blood pressure parameters in non-exercising and exercising subjects; * $P < 0.05$

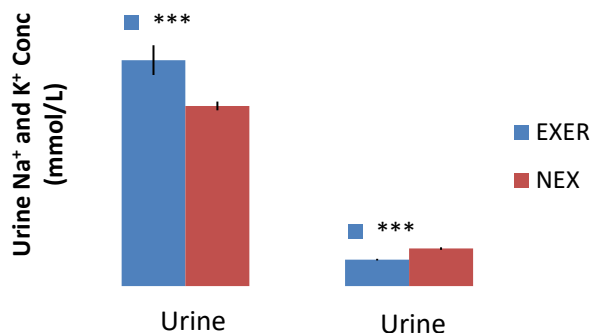


Figure 5: Comparison of urine Na^+ and K^+ concentration in non-exercising and exercising subjects. *** = $P < 0.001$

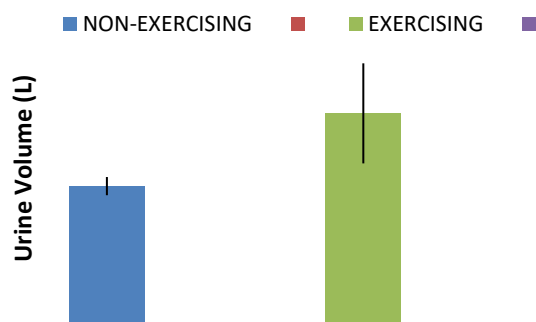


Figure 6: Comparison of urine volume in non-exercising and exercising subjects

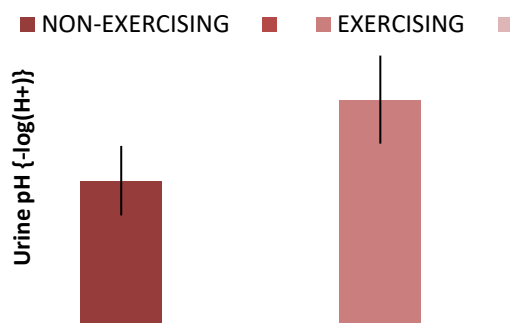


Figure 7: Comparison of urine pH in non-exercising and exercising subjects

Comparison Of Baseline Renal Function In Non-Exercising And Exercising Subjects: Urine Na^+ and K^+ concentration differed significantly in both groups (Fig. 5) with $P < 0.001$. There was no significant difference in the urine volume and urine pH of both groups (Fig. 6-7).

Table 5:

Effect of salt loading on the ventilatory function of non-exercising young men

Parameters	Before loading	After loadir	Change $\pm$ SEM	Remarks
FVC (Litre)	2.73 $\pm$ 0.15	2.72 $\pm$ 0.16	-0.003 $\pm$ 0.01	NS
FEV1 (Litre)	2.45 $\pm$ 0.13	2.40 $\pm$ 0.18	-0.05 $\pm$ 0.05	NS
FEV1%	90.49 $\pm$ 2.47	88.80 $\pm$ 2.36	-1.69 $\pm$ 0.11	NS
PEFR (L/MIN)	411.40 $\pm$ 22.7	391.20 $\pm$ 29.81	-20.20 $\pm$ 7.11	NS
SPO2	95.95 $\pm$ 0.55	95.10 $\pm$ 0.71	-0.85 $\pm$ 0.16	NS

Values are expressed as mean $\pm$ S.E.M

NS=Not significant

FEV1= forced expiratory volume in 1 sec; FVC = forced vital capacity; PEFR = Peak expiratory rate; SPO2 =oxygen saturation.

Effects Of Salt Loading on The Ventilatory Function Of Non-Exercising Subjects: Salt loading has no significant effect on the FEV1, FVC and PEFR of non-exercising subjects (Fig. 9-10), slightly reduced by -0.05 ± 0.05 L, -0.003 ± 0.01 L and -20.20 ± 7.11 L/min respectively (Table 5). Similarly, they also have no significant effect on their SPO2 and FEV1% (Fig. 8). Which fell slightly by $-0.85 \pm 0.16\%$ and $-1.69 \pm 0.11\%$.

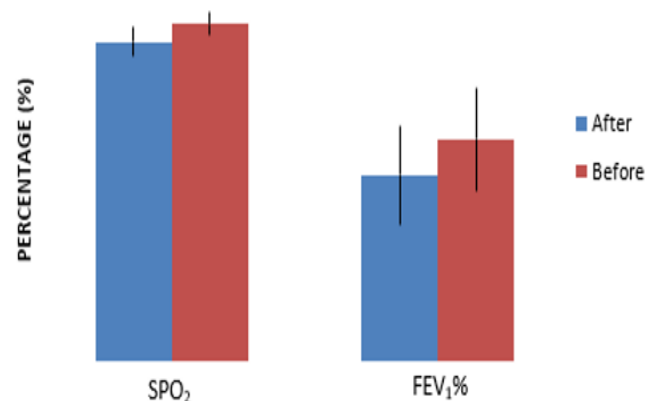


Figure 8: Effect of salt loading on the oxygen saturation (SPO2) and FEV1% in non-exercising subjects

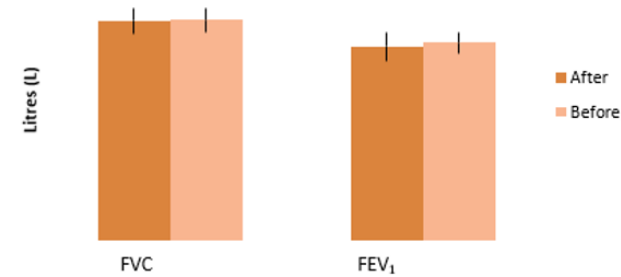


Figure 9: Effect of salt loading on the forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV1) in non-exercising subjects

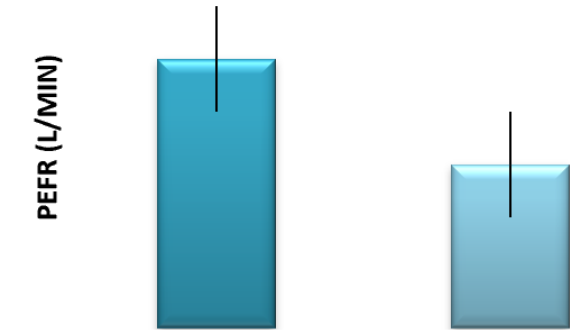


Figure 10: Effect of salt loading on the mean peak expiratory flow rate (PEFR) of non-exercising subjects

Effect of Salt Loading on the Blood Pressure Parameters of Non-Exercising Subjects: Salt loading have significant effect on the blood pressure parameters of non-exercising subjects (Fig. 11), causing significant increase ($P < 0.05$ and 0.01 respectively) in the SBP, DBP, MABP and PP of 18.00 ± 2.04 mmHg, 11.90 ± 1.52 mmHg, 6.20 ± 0.29 mmHg and 13.97 ± 1.98 mmHg respectively (Table 6) after five days of salt loading.

Table 6: Effect of salt loading on the blood pressure parameters of non-exercising subjects

Parameters	Before loading	After loading	Change $\pm$ SEM	Remark
SBP (mmHg)	107.20 ± 2.06	$125.30 \pm 4.10^*$	18.00 ± 2.04	SIG
DBP (mmHg)	70.00 ± 2.84	$81.90 \pm 2.66^{**}$	11.90 ± 1.52	SIG
PP (mmHg)	37.20 ± 3.58	$43.40 \pm 3.87^*$	6.20 ± 0.29	SIG
MABP (mmHg)	82.4 ± 3.26	$96.37 \pm 2.58^*$	13.97 ± 1.98	SIG

Values are expressed as mean $\pm$ S.E.M
SIG=Significant
*= $P < 0.05$, **= $P < 0.01$
SBP = systolic blood pressure; DBP = diastolic blood pressure; MABP = mean arterial blood pressure; PP = Pulse pressure

Effect of Salt Loading on Renal Function Parameters of Non-Exercising Subjects: Urine K^+ concentration increased significantly ($P < 0.05$) by 5.35 ± 0.36 mmol/L, urine Na^+ concentration decreased by -18.74 ± 0.28 mmol/L after five days of salt loading (Table 7). Salt loading has no significant effect on the urine volume, and urine pH (Fig. 13, 14). This fell slightly by 0.04 ± 0.01 L, -18.74 ± 0.28 mmol/L and -0.07 ± 0.06 respectively.

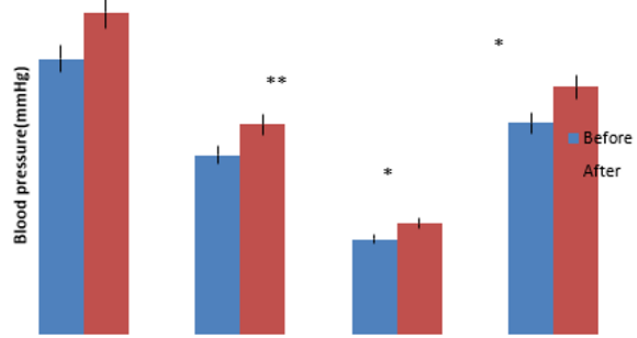


Figure 11: Effect of salt loading on the blood pressure parameters of non-exercising subjects. *= $P < 0.05$, **= $P < 0.01$

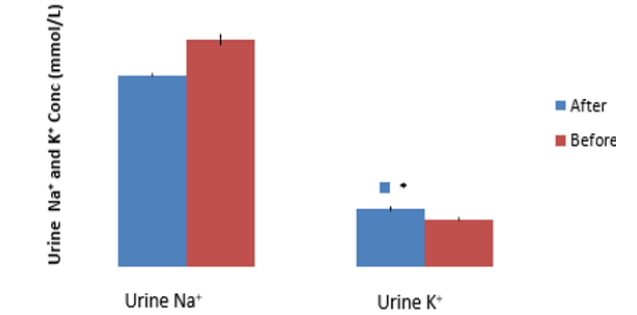


Figure 12: Effect of salt loading on the urine Na^+ and K^+ Concentration in non-exercising subjects. *= $P < 0.05$

Table 7: Effect of salt loading on the renal function of non-exercising young men

Parameters	Before loading	After loading	Change $\pm$ SEM	Remarks
Urine Na^+ concentration (mmol/L)	118.71 ± 2.89	$99.97 \pm 3.17^*$	-18.74 ± 0.28	SIG
Urine K^+ concentration (mmol/L)	24.72 ± 0.83	$30.07 \pm 1.19^*$	5.35 ± 0.36	SIG
Urine volume (Litres)	0.31 ± 0.02	0.28 ± 0.01	-0.04 ± 0.01	NS
Urine pH	6.23 ± 0.15	6.16 ± 0.21	-0.07 ± 0.06	NS

Values are expressed as mean $\pm$ S.E.M
SIG =Significant NS =Not significant; *= $P < 0.05$

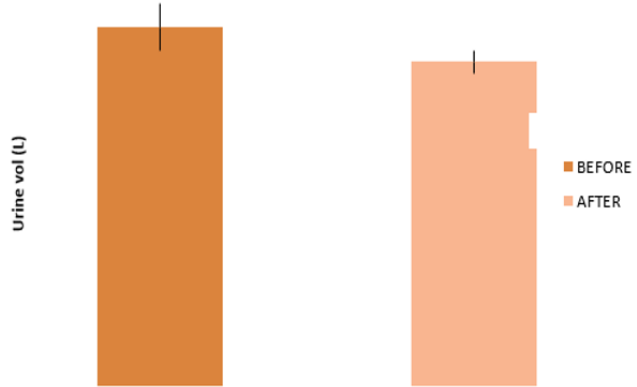


Figure 13: Effect of salt loading on the urine pH of non-exercising subjects

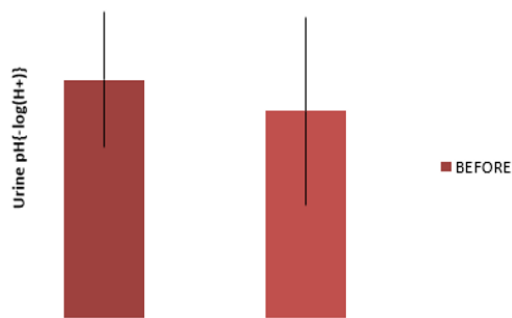


Figure 14:
Effect of salt loading on the urine volume of non-exercising subject

Effects of Salt Loading on The Ventilatory Function of Exercising Subjects: Exercising subjects experienced significant reduction ($P < 0.05$) in FVC, FEV1 and PEFR (Fig. 16-17) of -0.55 ± 0.04 L, -0.52 ± 0.03 L and -88.58 ± 1.14 L/min respectively (Table 8) after five days of salt loading. Salt loading had no significant effect on the FEV1% and SPO2 (Fig. 15). This fell slightly by -1.17 ± 0.06 % and -1.28 ± 1.06 % respectively.

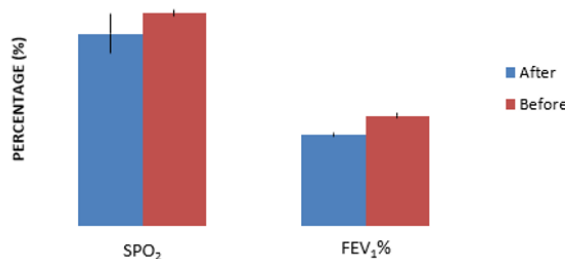


Figure 15:
Effect of salt loading on the oxygen saturation and fev1% of the exercising subjects.

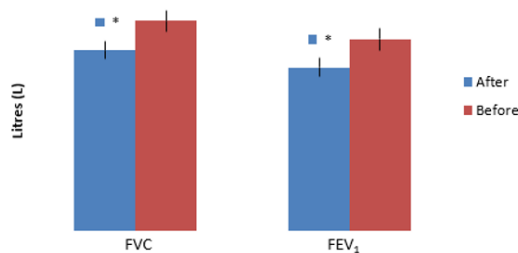


Figure 16:
Effect of salt loading on the forced vital capacity (FVC) and forced expiratory volume in 1sec (FEV1) of the exercising subjects
.*= $P < 0.05$.

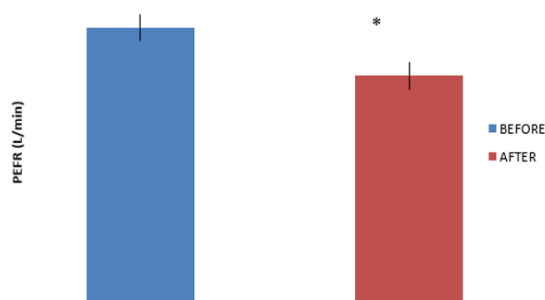


Figure 17:
Effect of salt loading on the peak expiratory flow rate (PEFR) of the exercising subjects. *= $P < 0.05$

Table 8:

Effect of salt loading on the ventilatory function of exercising subjects

Parameters	Before loading	After loading	Change $\pm$ SEM	Remarks
FVC (Litre)	3.93 ± 0.20	$3.38 \pm 0.16^*$	-0.55 ± 0.04	SIG
FEV1 (Litre)	3.58 ± 0.21	$3.06 \pm 0.18^*$	-0.52 ± 0.03	SIG
FEV1%	90.94 ± 0.17	89.77 ± 0.11	-1.17 ± 0.06	NS
PEFR (L/MIN)	511.30 ± 24.83	$422.72 \pm 25.97^*$	-88.58 ± 1.14	SIG
SPO2	97.40 ± 0.19	96.12 ± 0.25	-1.28 ± 1.06	NS

Values are expressed as mean $\pm$ S.E.M

SIG = Significant .NS =Not significant; *= $P < 0.05$

FEV1= forced expiratory volume in 1 sec; FVC= forced vital capacity; PEFR= Peak expiratory flow rate; SPO2 = oxygen saturation.

Table 9:

Effect of salt loading on the blood pressure parameters in exercising subjects.

Parameters	Before loading	After loading	Change $\pm$ S.E.M	Remarks
SBP (mmHg)	100.50 ± 1.53	$110.00 \pm 2.80^*$	9.50 ± 0.10	SIG
DBP (mmHg)	63.50 ± 3.14	70.00 ± 3.08	6.50 ± 0.06	NS
MABP (mmHg)	75.83 ± 2.01	83.33 ± 3.36	7.50 ± 1.35	NS
PP (mmHg)	37.00 ± 3.33	40.00 ± 3.72	3.00 ± 0.06	NS

Values are expressed as mean $\pm$ S.E.M

SIG =Significant NS =Not significant; *= $P < 0.05$

SBP =systolic blood pressure; DBP = diastolic blood pressure; MABP = mean arterial blood pressure; PP = Pulse pressure.

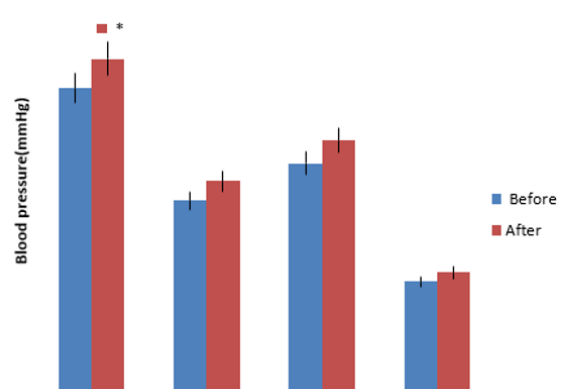


Figure 18:

Effect of salt loading on the blood pressure parameters in exercising subjects.
.*= $P < 0.05$.

Effect of Salt Loading on Blood Pressure Parameters of Exercising Subjects:

The Systolic blood pressure increased significantly ($P < 0.05$) from 100.50 ± 1.53 mmHg to 110.00 ± 2.80 mmHg. (Table 9) after salt loading. This is within the normal range in young adults. There was no significant increase in the Diastolic blood pressure, Mean arterial pressure and Pulse pressure after salt loading in the exercising subjects (Fig. 18). Which decreases slightly by

6.50 ± 0.06 mmHg, 7.50 ± 1.35 mmHg and 3.00 ± 0.06 mmHg respectively.

Effect of Salt Loading on Renal Function in Exercising Subjects: Urine Na⁺ concentration increased significantly (P < 0.05) from 148.96 ± 9.80 mmol/L to 160.23 ± 10.97 mmol/L after five days of salt loading (Table 10). Similarly, there was also a decrease in the Urine K⁺ Concentration, this fell significantly (P < 0.05) by -3.73 ± 0.21 mmol/L after five days of salt loading. Salt loading has no significant effect on their urine volume and urine pH. (Fig. 23-24).

Table 10:
Effect of salt loading on the renal function in exercising subjects

Parameters	Before loading	After loading	Change ±S.E.M	Remarks
Urine Na ⁺ concentration (mmol/L)	148.96 ± 9.80	160.23 ±10.97*	11.27 ± 1.17	SIG
Urine k ⁺ concentration (mmol/L)	17.44 ± 0.58	13.71 ± 0.37*	-3.73 ± 0.21	SIG
Urine volume (Litres)	0.47 ± 0.11	0.51 ± 0.10	0.01 ± 0.01	NS
Urine pH	6.58 ± 0.19	6.44 ± 0.17	-0.14 ± 0.03	NS

Values are expressed as mean ± S.E.M
SIG =Significant NS =Not significant; *= P < 0.05

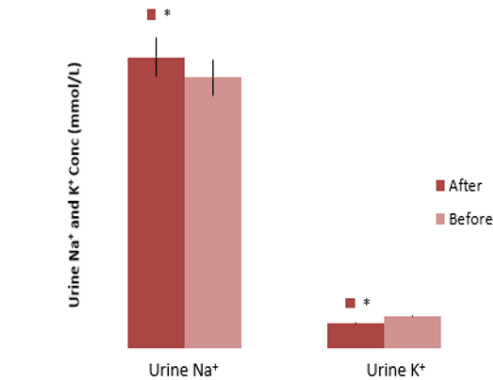


Figure 19:
Effect of salt loading on the urine Na⁺ concentration in exercising subjects; *= P < 0.05

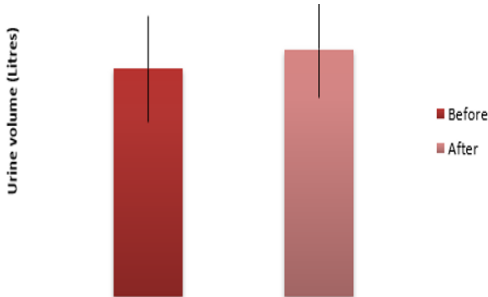


Figure 20:
Effect of salt loading on the urine volume of exercising subjects

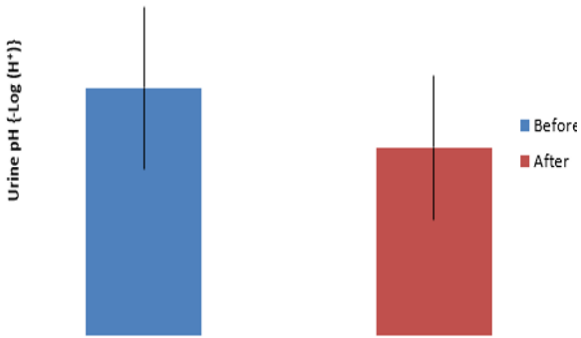


Figure 21:
Effect of salt loading on the urine pH of exercising subjects

Comparison of the Change (Δ) in Ventilatory Function in Non-Exercising and Exercising Subjects after Salt Loading: Salt loading caused a greater change in FVC (P < 0.05), FEV1 (P < 0.05) and PEFR (P < 0.05) in exercising subjects than the non- exercising subjects (Fig. 24-26) There was no significant change in the FEV1% and SPO2 of both groups.

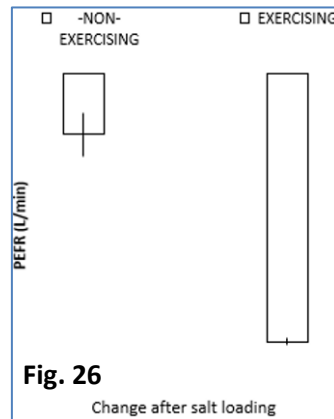
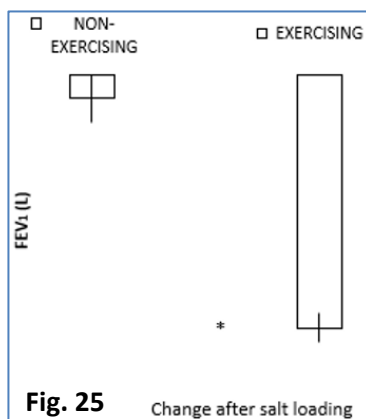
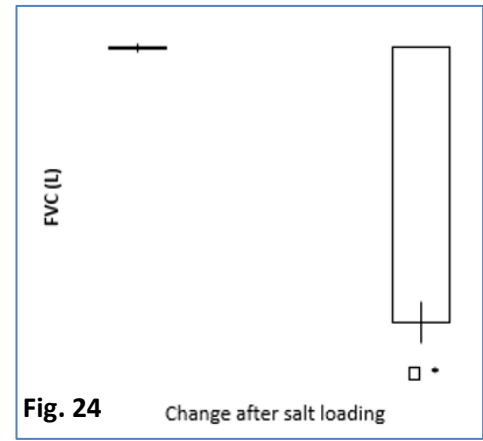
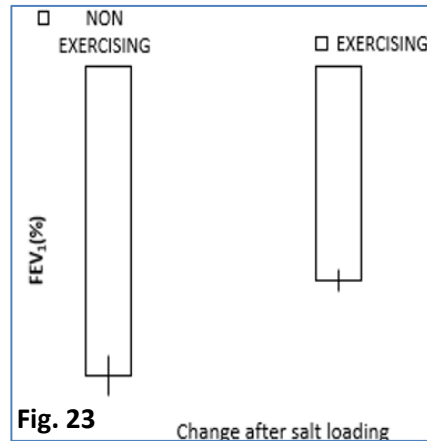
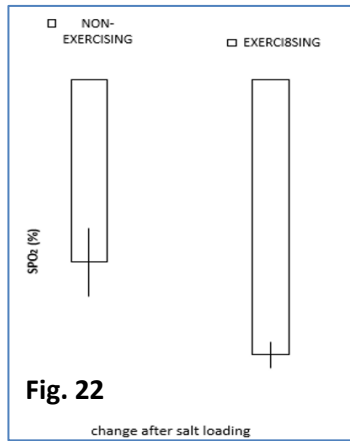
Table 11:
Comparison of the change (Δ) in ventilatory function of non-exercising (control) and exercising subjects after salt loading

Parameters	Non-Exercising Subjects	Exercising Subjects	Remark
FVC (Litre)	-0.003 ± 0.01	-0.55 ± 0.04*	SIG
FEV1 (Litre)	-0.05 ± 0.05	-0.52 ± 0.03*	SIG
FEV1%	-1.69 ± 0.11	-1.17 ± 0.06	NS
PEFR (L/MIN)	-20.20 ± 7.11	-88.58 ± 1.14*	SIG
SPO2 (%)	-0.85 ± 0.16	-1.28 ± 0.06	NS

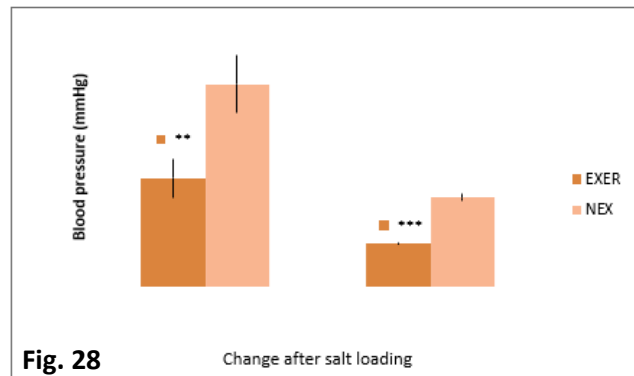
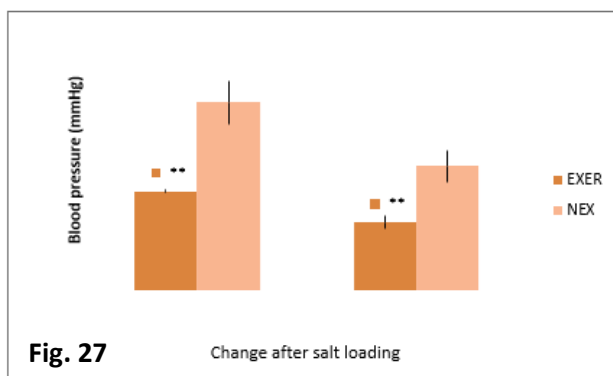
Values are expressed as mean ± S.E.M
SIG =Significant NS =Not significant; *= P < 0.05
FEV1= forced expiratory volume in 1 sec; FVC = forced vital capacity; PEFR= Peak expiratory flow rate; SPO2 = oxygen saturation.

Comparison of The Change (Δ) in Blood Pressure Parameters in Non-Exercising and Exercising Subjects After Salt Loading: Five days of salt loading caused a greater change in the systolic blood pressure (P < 0.01), diastolic blood pressure (P < 0.01), mean arterial blood pressure (P < 0.01) and the pulse pressure (P < 0.001) in the non-exercising subjects than the exercising subjects (Fig. 27, 28). This explains the powerful effect salt loading has on the blood pressure parameters of non-exercising subjects over their exercising counterpart.

Comparision Of The Change (Δ) In Renal Function In Non- Exercising And Exercising Subjects After Salt Loading: Salt loading caused a greater change in urine Na⁺ concentration and volume (P < 0.001and 0.05) in exercising subjects than the non- exercising subjects. Similarly, they also caused a greater change in the urine K⁺ concentration (P < 0.01) in non- exercising subject than the exercising subjects (Fig. 29).There was no significant change in the urine pH of both groups (Table 13).

**Figures 22 – 26**

Changes in oxygen saturation (SPO₂, **Figure 22**); FEV1%, **Figure 23**), FVC (**Figure 24**), (FEV1, **Figure 25**) and peak expiratory flow rate, PEFR, **Figure 26**) in non-exercising and exercising young men. * = P < 0.05

**Figures 27 and 28**

Changes in systolic and diastolic blood pressure, **Figure 27**) and changes in pulse pressure (PP) and mean arterial pressure (MAP) **Figure 28**) in non-exercising and exercising young men. * = P < 0.05

Table 12:

Comparison of the change (Δ) in blood pressure parameters in non-exercising and exercising subjects after salt loading

Parameters	Non-Exercising Subjects	Exercising Subjects	Remarks
SBP (mmHg)	18.0 $\pm$ 2.04	9.50 $\pm$ 0.10**	SIG
DBP (mmHg)	11.90 $\pm$ 1.52	6.50 $\pm$ 0.60**	SIG
MABP (mmHg)	13.97 $\pm$ 1.98	7.50 $\pm$ 1.35**	SIG
PP (mmHg)	6.20 $\pm$ 0.29	3.00 $\pm$ 0.06***	SIG

Values are expressed as mean $\pm$ S.E.M

SIG = Significant; ** = P < 0.01, *** = P < 0.001 respectively

SBP = systolic blood pressure; DBP = diastolic blood pressure;

MABP = mean arterial blood pressure; PP = Pulse pressure

Table 13:

Comparison of the change (Δ) in renal function in non-exercising and exercising subjects after salt loading

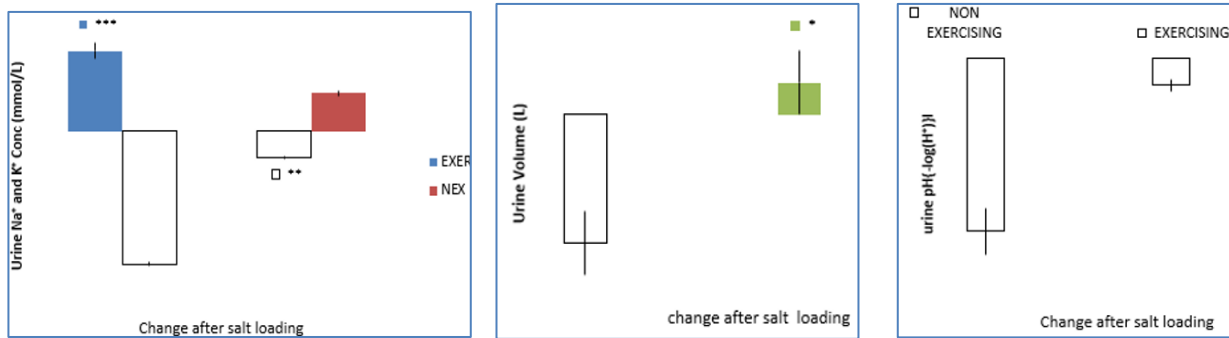
Parameters	Non-Exercising Subjects	Exercising Subjects	Remarks
Urine Na ⁺ conc. (mmol/L)	-18.74 $\pm$ 0.28	11.27 $\pm$ 1.17***	SIG
Urine K ⁺ conc. (mmol/L)	5.35 $\pm$ 0.36	-3.73 $\pm$ 0.21**	SIG
Urine volume (litre)	-0.04 $\pm$ 0.02	0.01 $\pm$ 0.01*	SIG
Urine pH	-0.07 $\pm$ 0.06	-0.14 $\pm$ 0.02	SIG

Values are expressed in mean $\pm$ S.E.M

SIG = Significant

** = P < 0.01, *** = P < 0.001 respectively

Exercise's impact on salt intake and health in young men



Figures 29 - 31:

Changes in urine Na⁺ and K⁺ concentration (Figure 29), urine volume (Figure 30) and urine pH (Figure 31) in non-exercising and exercising subjects.; ** = $P < 0.01$, *** = $P < 0.001$

DISCUSSION

Several investigators have demonstrated the possible influence of high dietary salt intake on the severity of asthma in asthmatics (Stephanie *et al.*, 2024). Large amount of experimental and clinical studies have also emphasized the critical role of sodium in the regulation of blood pressure and the implication of an abnormal sodium balance in the development of hypertension in humans (Lucia *et al.*, 2019). The aim of this study was to ascertain the effect of high salt intake on the blood pressure, Oxygen saturation, Lung function and renal function of young men who engage more often in exercising activities.

In this study, the effect of high salt intake on the blood pressure, lung function, and renal function of young men who regularly exercise was investigated. The results showed that before salt loading, exercising subjects had better lung function and lower blood pressure compared to nonexercising subjects. However, after salt loading, lung function and blood pressure worsened in exercising subjects while remaining unchanged in non-exercising subjects. This suggests that exercise may protect against the adverse effects of high salt intake on lung function and blood pressure. Additionally, salt loading affected renal function differently in exercising and non-exercising subjects, with exercising subjects showing increased urine sodium and potassium excretion compared to non-exercising subjects. The study highlights the complex interplay between salt intake, exercise, and various physiological parameters. Exercising subjects had better FVC values than the nonexercising subjects before a high salt intake. This observation is in line with the work of Prakash *et al.*, (2007) who made a similar observation in men engaging in physical exercise. A study by Douglas *et al.*, (1959) also made similar findings in men who engage in contact sports like basketball showing a significantly higher forced vital capacity compared to men that don't engage in such sport. Such observation was also reported in Athletes by Onadeko *et al.*, (1976), reporting a significantly higher FVC in athletes compared to non-athletes. Also, when nonexercising and exercising groups were compared, result showed higher FEV1 in exercising subjects as opposed to the report of Ayesha *et al.*, (2007) that did not observe any significant change in FEV1.

PEFR values were much higher in the exercising subjects than the non-exercising group. This observation is supported by the report of Nagarathna and Nagendia (1985) reporting an improvement in PEFR after two weeks of yoga

training. Likewise, FEV1 % and SPO2 were lower in nonexercising subjects than the exercising subjects as supported by the reports of previous studies observing significant difference in FEV1 % and SPO2 in actively exercising men. This might be due to the difference in their pulmonary capacity (Adegoke and arogundade, 2002).

Evidence that increased extracellular osmolality is associated with pro-inflammatory response is provided by studies manipulating serum osmolality using salt loading in rats, in which an increased serum osmolality resulted in an increase in the level of pro-inflammatory cytokines such as interleukin -1beta after rehydration (Summy-long *et al.*, 2006). This makes changes in osmolality after salt loading a modifier of the immune system. The possibility of exercise to intensify this effect by stimulating the release of more inflammatory mediators might be the mechanism via which exercise and high salt intake reduces lung function. Five days of salt loading decrease the value of FVC, FEV1 and PEFR more in the exercising subject than the nonexercising subjects, in the absence of any significant change in the SPO2 and FEV1% in both groups, owing to the augmentation of the pro-inflammatory effect of salt loading on ventilatory function by exercise training (Robert *et al.*, 2000).

Result obtained from this present study shows the value of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) were lower in the exercising subjects compared to the higher values in the non-exercising subjects. This may be due to the low fat deposition, elastic arterioles, increased shear stress, increased flow mediated release of nitric oxide (NO) and robust cardiovascular system in the exercising subjects (Hutcheson and Griffith, 1991).

In the exercising subjects five days of salt loading caused significant increase in systolic blood pressure (SBP) with no significant increase on their diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) compared to the significant increase in the blood pressure parameters of the non-exercising subjects. This agrees with the confirmation that salt loading elicits a sustained elevation in blood pressure i.e hypertension in humans (Fujita *et al.*, 1980) and experimental animals (Elias *et al.*, 2004). It also agree with the work of He and Macgregor (2002), who established a dose-response relationship between blood pressure and sodium intake between 50– 200mmol. The possibility of physical activity or exercise to prevent the significant increase in some of

the blood pressure parameters could be as a result of their protective effect to decrease the sensitivity of blood pressure to salt. This occurs through several potential mechanisms, such as reduction of insulin resistance, improvement of endothelial function and inhibition of sympathetic nervous system activity. Physical activity has been shown to be associated with reduced insulin resistance (Gill, 2007), and insulin resistance decreases renal sodium excretion, leading to extracellular fluid volume expansion and salt sensitive hypertension (Chen *et al.*, 2009). The mechanism by which physical exercise decrease insulin resistance are not yet fully explored (Gill and Malkova, 2006). It has been shown that vigorous physical activity result in physiologic adaptations of the skeletal muscle cells in adults. Some of the physiologic adaptations include increase capillary supply to the skeletal muscles (Hawley, 2002), an increase in the activity of enzymes of the mitochondrial electron transport chain, and a concomitant increase in mitochondrial volume and density (Hoppeler and Fluck, 2003). In addition, an increased substrate use (Holloszy and Coyle, 1984) with a decrease of carbohydrate oxidation and an increased muscle glucose transport might play a role (Holloszy, 2005). Indirectly, regular physical exercise may act by increasing lean body mass and concomitantly reducing body fat (Ruiz *et al.*, 2006). There is substantial evidence that resistance to insulin -mediated glucose disposal in non-diabetic individual is strongly associated with hyperinsulinemia (Hollenbeck and Reaven, 1987; Sun *et al.*, 2008). It has also been shown that the ability of insulin given acutely to enhance renal Na⁺ retention is maintained in individuals who are insulin resistant (Shimamoto *et al.*, 1994; facchini *et al.*, 1999). Consistence with this considerations is the current findings that the natriuretic response to NaCl loading was decreased in proportion to the degree of insulin resistance and presumably to the associated hyperinsulinemia. However, I believe this represents the demonstration that delayed Na⁺ balance and enhanced Na⁺ retention occurs in healthy, non-obese, insulin – resistant individuals to cause the change in blood pressure associated with sodium chloride loading. Before salt loading, exercising subjects had a higher urine Na⁺ concentration and volume than the nonexercising subjects. This could be as a result of their augmented basal and agonist induced nitric oxide release (Pritchard *et al.*, 1994) to modulate the excretory response to changes in arterial pressure via the pressure natriuresis and diuresis mechanism. A mechanism that have being demonstrated by separate studies to be reduced by acute (Majid *et al.*, 1993), short term (guarasci and kline, 1996) or long term (Atucha *et al.*, 1996) inhibition of nitric oxide synthesis. Another possible mechanism that could explain this difference is the inhibition of tubular reabsorption of sodium (Na⁺) and water by nitric oxide in several segments of the nephron (Garvin and Oritz, 2002), which may be induced by changes in renal interstitial pressure (Garcia and roman, 1989), and intra -renal medullary hemodynamics (Cowley, 1992).

Urine Na⁺ concentration and volume increased significantly in exercising subjects. In non-exercising subjects, salt loading significantly decreased urine Na⁺ concentration, causing a slight reduction in urine volume.

Similar observation was found in Dahl- sensitive rats (DS) and Dahl resistant rats (DR) by Hustel *et al.* (1996), who reported greater Na⁺ reabsorption by the inner medullary collecting duct cell from Dahl salt sensitive rat (DS) than those from Dahl salt resistant rats (DR).

In conclusion, five days of salt loading in non-exercising and exercising young men. Worsens pulmonary function more in exercising subjects than the non-exercising subjects in the absence of any effect on FEV1% and SPO2. Increase blood pressure significantly in the non-exercising subjects than the exercising subjects. Increase urine Na⁺ concentration and 12 hours urine volume in exercising subjects compared to the decrease in the non-exercising subjects. Decrease urine k⁺ and pH in the exercising subjects compared to the increase in the non-exercising subjects.

REFERENCES

- Andrea G, Lucia .S, Paolo.C.; (2019). Sodium intake and hypertension; Nutrient; vol 11, pg 1970
- Adegoke OA & Arogundade O (2002). The effect of chronic exercise on lung function and basal metabolic rate in some Nigerian athletes. *African journal of biomedical research* 5:9-11.
- Akita S, Sacks FM, Svetkey LP, Conlin PR, Kimura G: For the DASH sodium trial collaborative research group (2003). Effects of the dietary approach to stop hypertension (dash) diet on the pressure natriuresis relationship. *Hypertension* 42..
- America Heart Association, 2010 Dietary Guideline “2010 Dietary Guideline; *American Heart Association*, 2009. American heart Association (2010). Dietary guideline “Dietary guideline” *American heart Association*. 2009.
- Australia: Better Health Channel: Betterhealth.vic.goo.all, 2011.
- Bankir L, Bochund M, Maillard M, Bovet P, Gabriel and burner M (2008). Nighttime blood pressure and nocturnal dipping are associated with daytime urinary sodium excretion in African subjects. *Hypertension* 51;891-898.
- Chen J, Gu D, Huang J. (2009). Metabolic syndrome and salt sensitivity of blood pressure in non-diabetic people in china; a dietary intervention study. *Lancet* vol 373 (9666):829-835.
- Coetzee A. and Swanepoal C (1990): The Oxyhemo- globin dissociation curve before, during and after cardiac surgery. *Scandinavian Journal of clinical and Laboratory Investigation*. vol 203, Page 149 – 153, .
- Coruzzi P, Parati G, Brambilla L, brambilla V, Gualerzi M, Novarini A, Castiglioni P and jeunemaitre X. (2005). Effect of salt sensitivity bon neural cardiovascular regulation of essential hypertension. *Hypertension*; 46; 1321-1326
- Dumler F (2008). “Dietary Sodium Intake and arterial Blood pressure” *Journal of renal nutrition*, Vol 19 page 57 – 60,
- Elias S, Dina T, Sofola O.A. (2004). Carotid chemoreceptor reflex following dietary salt loading in rats. *Blood pressure* 13 (5):316-320.
- EL-sayed H and Hainsworth R (1996). Salt supplementation increases plasma volume and orthostatic tolerance in patients with unexplained syncope; *heart* 75;134-140.
- End of War on Salt; Scientific American, 2011.
- European salt producers Association. EU.salt.com, 2011.
- Franco V & Oparil S (2006). Salt sensitivity, a determinant of blood pressure, cardiovascular disease and survival. *Journal of American college of disease*; 25; 245s-255s.

- Fukunda M, Goto B, Kimura G (2006). Hypothesis on renal mechanism of non-dipper pattern of circadian blood pressure rhythm. *Med. Hypotheses* 67; 802-806.
- Gill JM & Malkova D (2006). physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. *Clin sci* (London); 110:409-425.
- Gill JM. (2007). physical activity, cardiorespiratory fitness and insulin resistance: a short update. *Current opinion lipidology*; 18(1):47-52.
- Gregg, I. Harold MC (2004). On food and cooking; Scribner 2nd Edition Page 642.
- Haugen E & Nath KA (1999). The involvement of oxidative stress in the progression of renal injury. *blood purif* 17:5865.
- He FJ & Macgregor GA (2002). Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials: implications for public health. *J. human hypertension* 16:761-770.
- He FJ & Macgregor GA (2007). Salt, blood pressure and cardiovascular disease. *Curr opin cardiol*; 22:298-305. Health Canada "Health Living Sodium: Healthy Living Health Canada, 2008.
- Higashi Y, Sasaki S, Nakagama K (2001) Sodium chloride loading does not alter endothelium dependent vasodilation of fore arm vasculature in either salt sensitive and salt resistance patient with essential hypertension, hypertension research vol 24, page 711-716.
- Holloszy JO. (2005). Exercise -induced increase in muscle insulin sensitivity. *J appl physiol* ;99:338-343.
- Hoppeler H & fluck M (2003). Plasticity of skeletal muscle mitochondria: Structure and function. *Med sci sport exerc*; 35:95-104.
- Howley JA (2002). Adaptations of skeletal muscle to prolonged, intense endurance training. *Clin Exp pharmacol physiol*; 29:218-222.
- Hsiung yen, Hua-yang J, Rong sheu y, Mei lee Y, An-ding Y. (1995). Chronic exercise enhances endothelial mediated dilation in spontaneously hypertensive rats. *Life sci* 57;22052213.
- Husted RF, Takahashi T, strokes JB. *American journal of physiology*. vol 271:F1029-F1036.
- Hutcheson IR & Griffith TM (1991). Release of endothelium derived relaxing factor is modulated both by frog and amplitude of pulsatile flow. *Am j physiol* 261:H257- H262.
- Institute for Health Metrics and Evaluation (IHME) diet high in sodium. Global Burden of Disease; 2019 (https://www.healthdata.org/results/gbd_summaries/2019/diet-high-in-sodium-level-3-risk).
- International Codex Alimentarius Standard for Food Grade Salt (ICAFS), 2011.
- INTERSALT (1988) An international study on electrolyte excretion and blood pressure results of the 24hr urinary sodium and potassium excretion. *BMJ*; 297:311-328.
- Jacquish CE, Rao DC, Hixson JE, Chen JC, Duan X, Huang JF, Chen CS, Kelly TN, Bazzano LA, and whelton PK; for the gensalt collaborative research group (2009). Gender difference in blood pressure responses to dietary sodium intervention in the Gensalt study. *journal of hypertension*; 27;48-54.
- Jaja, S. I, Opesanwo O, Mojiminiyi F. B. O. And Kehinde M. O. (2000). Lung Function, Haemoglobin and Irreversibly Sickled Cells in Sick Cell Patients. *West Afr. J Med* 19(3): 225-229.
- James V & Livingstone (2005). Agriculture and soil pollution: New research Nova publishers, page 45, .
- Kaplan NM (2000). The dietary guidelines for sodium ; should we shake it up/ no. *American journal of clinical nutrition*.
- Khalid RA (2006). Molecular mechanism linking salt to hypertension; New molecular target adds more spice. *American journal of physiology*, vol 290;R-509-R-513.
- Kim, HL., Park, S.M., Cho, I.J. et al. (2023). Standardized protocol of blood pressure measurement and quality control program for the Korea National Health and Nutrition Examination Survey. *Clin Hypertens* 29, 28
- Kocks P. 1. And Scott, D. D. (1990). Ammonia Salt Health Hazard in Work Place. *South Afr. Med. J.* 78: 45.
- Mathers CD and loncor D (2006) Projections of global mortality and burden of disease from 2002. plus med volume 3. page 11.
- Medici T.C and W. Vetter (2009). Bronchial asthma and kitchen salt. *Schweiz Med. Wochenschr.* 12 Life Hugger. *Medical Memories* Page 12-19.
- Meneton P, jeunemaitre X, wardner DE, HE and Macgregor G (2005). Links between dietary salt intake, renal salt handling, blood pressure and cardiovascular diseases.; *physiology revision* ;85;679-715.
- Nagarathna R, Nagendra HR. (1985). Yoga for bronchial asthma: a controlled study. *British medical journal*; 291;1077.
- Nakayama T, soma M, Takahasi Y, Rehemudula D, kanmatsuse K, and Furuya K (2009); functional deletion mutation of the 5'-flanking region of type A human natriuretic peptide and association with essential hypertension and left ventricular hypertrophy in the Japanese. *Circulation and respiration* ;vol 86;841-845.
- Nathell I, Nathell M, malmberg P (2007). COPD diagnosis related to different guidelines and spirometry techniques. *respiratory research* vol 8, page 89
- National lung and blood institute (NLBI); what is COPD, 2010
- Noncommunicable Diseases Data Portal. Geneva: World Health Organization; 2022 (<https://ncdportal.org/>). O'bryne P (1997). Exercise induced bronchoconstriction; elucidating the role of leukotrienes and prostaglandins. *Pharmacotherapy* vol 17 31s-38s.
- Obasohan AO, Okoh VA, Onyia KA and isiah AO (1992). Salt taste threshold in normotensive and hypertensive Nigerians; *tropical cardiology* vol 18;183-187.
- Odunaga, A. C, Jaja S. I. & Ojo, G.O (1989). Some Ventilatory Parameters In Well-Trained Nigerians Athletes. Oduntan S. A. (1970). Spirometric Studies of Normal and PreOperative Patients in Nigeria. *Afr. 1. Med Sci.* 1: 79-84.
- Ortiz PA & Garvin JL (2002). Role of NO in the regulation of nephron transport. *Am. j renal physiol* 282:F777-784.
- Osim E. E & Esin R. A. (1996). Lung Function Studies in Some Nigerian Bank Worker. *Central Afr. 1. Med.* 42(2): 43-45.
- Osim E. E, Uorn S. E. & Antai A. B. (2004): Symptoms And Lung Function Values In Nigerian Men and Women Exposed To Dust Generated From Crushing Of Giant Rocks in Calabar, *Nig. 1. Physiol. Sci.* 19(1-2): 41-47.
- Parakash S, Meshiam S, ramilekhar U. (2007). Athletes, yogi's and individuals with sedentary lifestyles, do their lung function differs ? *indian j. physiol pharmacol* ;51(1);76-80.
- Paterick TF & fletcher G. (2001). Endothelial function and cardiovascular prevention; role of blood lipids, exercise and other risk factors. *Cardiol* ;vol 9 (5): 282-286.

- Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz D, Sheps SG, and Rocella EJ (2005). Recommendations for blood pressure measurement in humans and experimental animals .Part1;blood pressure measurement in humans. A statement for proffessionals from the subcommittee of professional and public education of the American heart association council on high blood pressure research; *Hypertension*;45;142-161.
- Rasmussen MS, Simonsen JA, Sandgaard NCF, HoilundCarlsen PF and Bie .P (2003).Mechanisms of acute naturesis in normal humans on low sodium diet. *Journal of physiology*; vol 546;591-603.
- References on food salt and health issue's; salt institute. 2009
- Renner J. K, Oduwale A. O. And Jaja S. I. (1999). Effect of Stance on Peak Expiratory Flow Rate: Nig. J Clin. Pract. 2(2): 56-58.
- Sacks FM, Sverkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obsarzanek E, Conlin PR, Miller ER, SimmonsMorton DG, karanja N , Lin PH and DASH sodium collaborative research group (2001).Effect of blood pressure on reduced dietary sodium and dietary approach to stop hypertension (DASH) DIET. *England journal of medicine*.344;3-10.
- Safety Data for Sodium Chloride: the Physical and theoretical chemistry laboratory of Oxford University, 2005.
- Sanders PW (2008). Salt sensitivity: it is not always in the genes. *Hypertension* 51; 823-824.
- Scientific Advisory Committee on nutrition (SACN) (2003). Salt and Health; Page 3.
- Scientific Advisory Committee on Nutrition (SACN) Salt and Health Page 18.
- Sessa WC, Pritchard K, Seyedi N (1994). Chronic exercise in dogs increases coronary vascular NO production and endothelial NOS gene expression. *Clin res* 74;349-353.
- Shahidi *et al* (2005). Asian functional foods: *Boca Raton*: Page 575,
- Sofola OA (2004). Salt, blood pressure and cardiovascular changes in humans and experimental studies. A review *Nigerian medical practice*;46;22-26.
- Summy-long JY, Phillips TM, Pruss A (2006). Response of interleukin 1beta in the magnocellular system to salt loading. *Journal of neuro endocrinol* vol 18, Page 926-937.
- Summy-long JY,Hu S,Pruss A,Chen X, Philps TM.(2006).Response of interleukin -1b in the magnocellular system to salt loading. *Neuroendocrinol* 18:926-937.
- Glew R. H., H. Kassam, J. Vander Voort, P. A. Agaba, M. Harkins, D. J. VanderJagt. (2004): Comparison Of Pulmonary Function Between Children
- Volmer WM ,Sacks FM, Ards J, Appel LJ, bray GA, SimmonsMorton DG, Conlin PR, Svetkey LP, Erlinger TP, Moore TJ, Karanja N (2001). For the DASH-sodium trial collaborative research group. Effects of diet and sodium inta8ke on blood pressure; subgroup analysis of the DASH-sodium trial. *Ann intern medicine* 135;1019-1028
- Wang H, Carretero OA, Garvin JL.(2003). Inhibition of apical Na⁺/H⁺ exchanger on the macula densa cells auguments tubuloglomerular feedback .hypertension 41.
- Weinberger MH (1996).salt sensitivity of blood pressure in humans .*Hypertension* 27;481-490
- William F Ganong; (2006). Review of medical physiology. Twenty- third edition.
- Williams and Wilkins (2007). lippincott's illustrated review biochemistry 4th edition Page 24-35.

Full length Research Article

Cross-Examination and Comparison of Effects of Nutritive and Non-Nutritive Sweeteners on Male Reproductive Health Using Wistar Rat Model

***Aikpitanyi I.¹, Okeke O.C.¹, Ogbara F.F.², Ayunku E.A.¹, Nweke S.M.³,
Iyamu M.¹, Amaku HA.¹**

¹Department of Physiology, School of Basic Medical Science, Oba Okunade Sijuade College of Health Science, Igbinedion University Okada, Edo State, Nigeria.

²Department of Physiology, School of Basic Medical Science, University of Benin, Edo state, Nigeria.

³Department of Anatomy, University of Benin, Edo State, Nigeria

Summary: Sucrose and saccharin are prevalent sweeteners in today's diet and play crucial roles in global food and beverage consumption. The intake of sucrose, a natural sweetener, alongside saccharin, an artificial alternative, has raised various health concerns related to its effects on reproductive health. This study specifically focuses on how these sweeteners influence male reproductive parameters, including sperm quality, hormonal levels (such as follicle stimulating hormone {FSH}, luteinizing hormone {LH}, and testosterone), reproductive gene expression, and the functionality of reproductive organs, in animal models (in vivo) and by also identifying the mechanisms through which these substances impact male reproductive health. Thirty male Wistar rats, each weighing 180–200 g, were randomly assigned to three groups: Group one served as the control, Group two received sucrose (100 mg/kg), and Group three was administered saccharin (5 mg/kg). The findings indicated that the levels of follicle stimulating hormone, luteinizing hormone, testosterone, and steroidogenic acute regulatory protein (StAR) significantly decreased in the sucrose and saccharin groups compared with those in the control group. Additionally, the testicular antioxidant malondialdehyde levels increased relative to those in the control, whereas superoxide dismutase and catalase levels were significantly decreased. Furthermore, the sperm profile—including sperm count, motility, and viability—was significantly altered compared with that of the control group. These results suggest that both sweeteners can negatively impact reproductive function by diminishing testicular antioxidant activity, altering sperm characteristics, and inhibiting StAR, ultimately leading to testicular damage, with the sucrose exhibiting a more pronounced negative effect on reproductive function.

Keywords: Sucrose, Saccharin, Sweeteners, Hormone levels, Sperm quality. Wistar rats

*Authors for correspondence: aikpitanyi.ikponmwosa@iuokada.edu.ng, Tel: +234-8146116220

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INTRODUCTION

The evolution of the food industry has resulted in a diverse array of sweeteners available to consumers. These sweeteners offer a delightful taste, which can be either energy-providing (nutritive) or calorie-free (nonnutritive) (American Dietetic Association, 2004). In the early stages of human evolution, *H. sapiens* fulfilled its craving for sweetness by consuming naturally sweet foods such as fruits and vegetables (Reed and McDaniel, 2006). As the food industry has expanded, it has enabled the mass production of refined sugar derived from sugar beets and sugarcane (Arshad *et al.*, 2022). Today, the market is flooded with a variety of sugar substitutes to meet the growing demand for sweeteners (ADA, 2004).

Sweeteners can be classified into two categories: nutritive and nonnutritive. Nutritive sweeteners supply

energy, whereas nonnutritive sweeteners offer sweetness without contributing any calories (ADA, 2004; Fitch and Keim, 2012).

The male reproductive system comprises internal structures, such as the testes, epididymis, vas deferens, and prostate, as well as external structures, such as the scrotum and penis. These components are richly supplied with blood vessels and include various glands and ducts that facilitate reproduction, sperm storage, and ejaculation while also producing androgens essential for male development (Tiwana and Leslie, 2017). The key hormones involved in regulating these processes include follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted by the anterior pituitary gland, along with gonadotropin-releasing hormone (GnRH), which is produced by the hypothalamus. Together, these hormones play crucial roles in promoting and sustaining sexual

development and function in males through the hypothalamic–pituitary–gonadal axis (Tiwana and Leslie, 2023).

In the mid-19th century, sugar was regarded as a "pure white and deadly" substance, largely because of its association with various harmful effects (Handelsman, 1989). While there is insufficient evidence directly linking sucrose intake to toxicity, numerous studies indicate that sugar consumption may be associated with a range of negative health outcomes (Howard and Wylie-Rosett, 2002).

Artificial and natural sweeteners have a long history of use and have become staple components of many diets. Anecdotal evidence suggests that these sweeteners may influence various organ systems. Increased dietary intake of sweeteners has been linked to the development of several health issues, including autoimmune and cardiovascular diseases, cancer, dental caries, gallstones, premature aging, appendicitis, depression, hyperactivity, and various metabolic disorders (Howard and Wylie-Rosett, 2002). Although these compounds are often marketed as low-calorie or calorie-free sweeteners, some studies indicate that they may lead to sperm abnormalities, alterations in sperm biochemical properties, and reduced sperm motility (Burke and Small, 2015). Changes in sperm morphology could hinder the ability of a sperm to fertilize an egg, thereby impacting fertility (Moretti et al., 2022). Additional research has shown that artificial sweeteners can affect spermatogenesis; for example, sodium saccharin significantly reduces testosterone levels in treated rats (Helal et al., 2019). This study aims to fill this knowledge gap by examining the differential impacts of sucrose and saccharin on sperm quality, hormone levels, testicular morphology, and reproductive organ function in male Wistar rats. By clarifying the specific effects of these two sweeteners on male reproductive health, this research will offer valuable insights into the potential risks associated with their consumption and aid in informed decision-making regarding dietary practices and public health policies.

MATERIALS AND METHODS

Duration and setting of the study: The study was conducted in the Department of Physiology Laboratory, Igbinedion University Okada, from May 2024 to July 2024. The animals were handled in accordance with the Guiding Principles for Research as recommended by the Declaration of Helsinki and the Guiding Principles for the Care and Use of Laboratory Animals.

Procurement and maintenance of animals: The experimental animals used in this study were thirty (30) healthy male Wistar rats weighing between 180 g and 200 g. The animals used were obtained from the central animal house of Igbinedion University Okada. The animals were provided with proper housing and were kept at a standard temperature. As a precaution against infection, bedding was changed daily, and the cages were washed every day. The animals were provided with water and feed on daily (Akhter et al., 2023).

Experimental design, animal treatment and collection of tissues: The 30 male Wistar rats were divided into three (3) equal groups at random, each containing ten rats, weighed on the day of arrival, before and after treatment, and properly labelled with markers for easy identification. The animals were treated daily for 64 days as follows: Group one: control (distilled water), Group two: sucrose (100 mg/kg), and Group three: saccharine (5 mg/kg) via oral gavage. The animals were anaesthetized with chloroform on the last day (Akpantah et al., 2003). Blood samples were obtained via cardiac puncture, and plasma was obtained for hormone (testosterone, FSH, LH) assays. The testis with the epididymis was dissected, and caudal epididymal sperm were analysed. The right testes were homogenized, and the resulting supernatant was used for superoxide dismutase (SOD), catalase activity, malondialdehyde (MDA) and StAR gene investigations. The left testes were fixed in Bouin's fluid for histological examination.

Hormonal profile investigations: Blood samples were obtained via cardiac puncture into plain bottles and spun at 3,500 rpm for 10 minutes in a cold centrifuge and samples were assayed for follicle-stimulating hormone, luteinizing hormone and testosterone using their respective Calbiotech ELISA kits as described by Okeke et al. (2023).

Gene expression Study

Isolation of Total RNA: Total RNA was isolated from tissue samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNase I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

cDNA conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Elekofehinti et al., 2020).

PCR amplification and agarose gel electrophoresis:

Polymerase chain reaction (PCR) for the amplification of Steroidogenic Acute Regulatory Protein (StAR) was carried out with OneTaqR2X Master Mix (NEB) using its primer (Inqaba Biotech, Hatfield, South Africa). PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing for 30 s and extension at 72 °C for 60 s) and ending with final extension at 72 °C for 10 min. The amplicon was resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of the gene, and quantification of band intensity was done using "image J" software (Olumegbon et al., 2022).

Antioxidant activity: The obtained supernatant was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels (Oyeyemi et al., 2020).

Sperm profile: The sperm count, motility, and viability assessment were performed immediately after the animals were anaesthetized. The right epididymis was excised immediately with care to minimize blood contamination and placed into a prewarmed (37°C) Petri dish containing two mL of a phosphate-buffered saline solution (pH 7.4). The spermatozoa were assessed for total sperm count and the percentages of motile, progressive, nonprogressive and immotile sperm cells (Tannenbaum *et al.*, 2003).

Histological Investigations: The testes and epididymis sections were subjected to Harri's hematoxylin and eosin staining, and the nuclei were stained blue, while the cytoplasm was stained pink, following the method described by Akpantah *et al.* (2003).

Statistical analysis: GraphPad Prism version 8.0.1 was used for the data analysis. One-way analysis of variance (ANOVA) was used to determine the differences between means among the groups, followed by Tukey's post hoc test. The results are expressed as the means $\pm$ SEMs. Differences between means were considered statistically significant at $p < 0.05$.

RESULTS

Hormones: Compared with the control, the oral administration of sucrose and saccharine significantly reduced the FSH, LH and testosterone levels ($P < 0.05$) (Table 1).

Testicular oxidant and antioxidant enzymes: The testicular MDA concentration was significantly increased in the sucrose and saccharin groups compared with the control

group ($p < 0.05$). Testicular catalase and superoxide dismutase were significantly decrease in sucrose and saccharine group when compared to control group ($p < 0.05$) (Figs. 1-3).

Sperm profile: Sperm count and viability (Figure 4 and 5) were significantly reduced in male Wistar rats treated with sucrose and saccharine compared with the control group ($P < 0.05$).

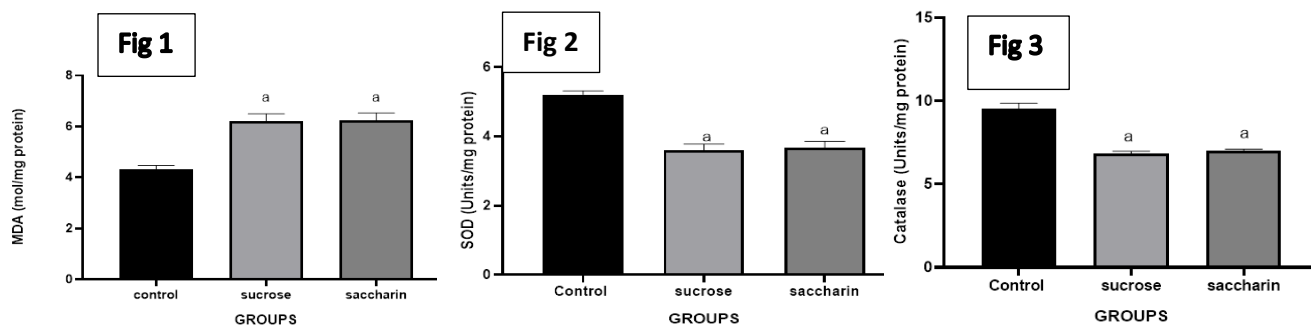
Table 1:

Effects of natural and artificial sweeteners on reproductive hormone levels in male Wistar rats.

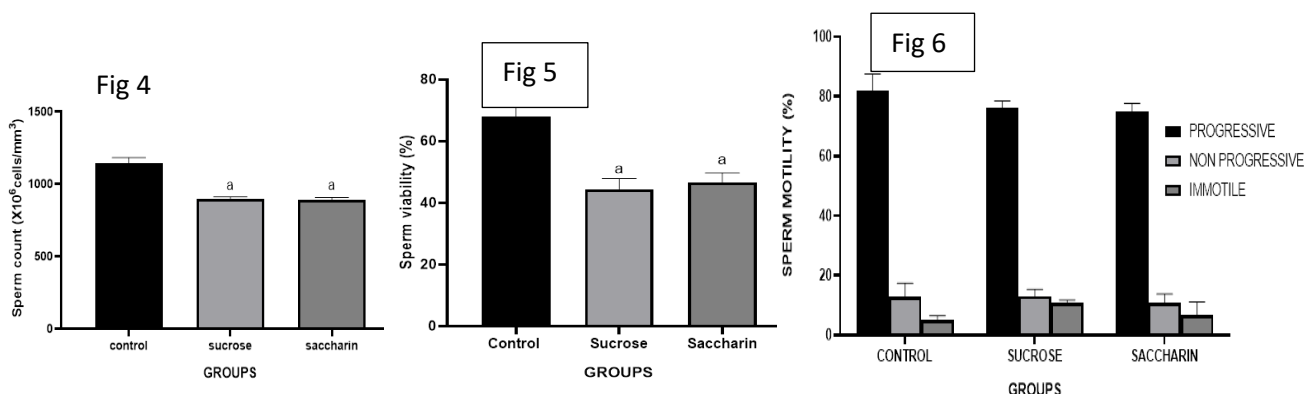
Group	FSH (U/L)	LH (U/L)	Testosterone (ng/ml)
Control	0.173 $\pm$ 0.002	0.623 $\pm$ 0.012	0.328 $\pm$ 0.008
Sucrose	0.030 $\pm$ 0.004 ^a	0.120 $\pm$ 0.015 ^a	0.120 $\pm$ 0.004 ^a
Saccharin	0.110 $\pm$ 0.004 ^{a,b}	0.180 $\pm$ 0.001 ^{a,b}	0.198 $\pm$ 0.006 ^{a,b}

(a, b) ($p < 0.05$) significantly different from the control group and sucrose group, respectively.

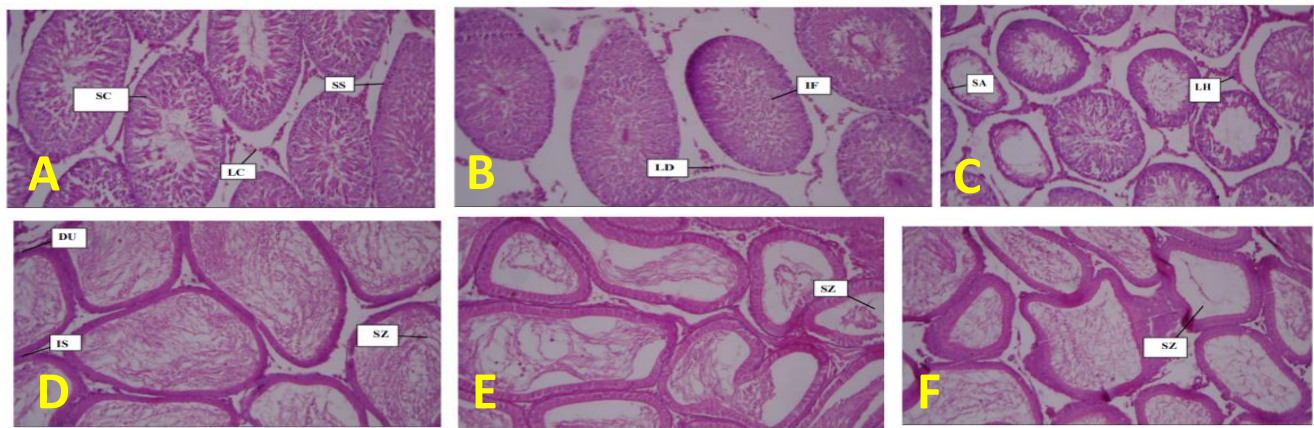
Testicular and epididymal histology: The control group (Plate 1A) showed tubules lined with normal spermatogenic series, Sertoli cells and interstitial Leydig cells, whereas the sucrose group (Plate 1B) showed Leydig cell degeneration, a tubular lumen packed with immature forms, and the saccharine group (Plate 1C) presented patchy spermatogenic arrest and Leydig cell hyperplasia. The epididymis of the rats in the control group presented normal ducts, dilated spermatozoa, and interstitial spaces. The epididymis of the rats in the sucrose group presented remarkable ductal depletion of spermatozoa, whereas the epididymis of the rats in the saccharine group presented patchy ductal depletion of spermatozoa.



Figures 1, 2, and 3: Effects of sucrose and saccharin on SOD, MDA and catalase in male Wistar rats. (a, b) ($p < 0.05$) significantly different from the control group and sucrose group, respectively.

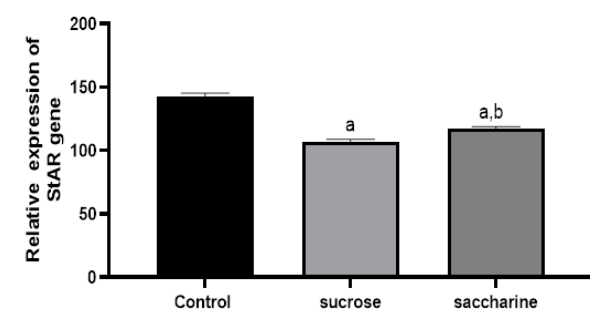
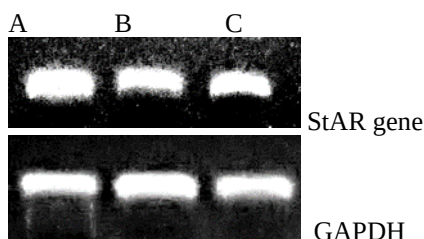


Figures 4-6: Effects of sucrose and saccharin on sperm count, viability and motility in male Wistar rats. (a, b) ($p < 0.05$) significantly different from the control group and sucrose group, respectively.

**Plate 1**

- A. Rat testes, group A (control), showing tubules lined with a normal spermatogenic series (SS), Sertoli cells (SCs) and interstitial Leydig (LC) cells: H&E $\times 100\times$
- B. Rat testes, group B (sucrose), showing Leydig cell degeneration (LD) and a tubular lumen packed with immature forms (IF): H&E 100X.
- C. Rat testes, group C (saccharine), showing patchy spermatogenic arrest (SA) and Leydig cell hyperplasia (LH): H&E 100X.
- D. Rat epididymis, group A (control), showing the following: normal ducts (DUs), dilated with spermatozoa (SZs), and interstitial space (IS): H&E $\times 100\times$.
- E. Rat epididymis, group B (sucrose), showing remarkable ductal depletion of spermatozoa (SZ): H&E 100X.
- F. Rat epididymis, group C (saccharine), showing patchy ductal depletion of spermatozoa (SZ): H&E 100X.

Testicular StAR gene expression: A significant reduction in testicular StAR gene expression was seen in the rats treated with sucrose and saccharine compared with the control group ($P < 0.05$), and a significant increase was observed in the saccharine group compared with the sucrose group ($p < 0.05$).

**Figure 7:**

Effects of sucrose and saccharin on the StAR gene in male Wistar rats.

(a, b) ($p < 0.05$) significantly different from the control group and sucrose group, respectively.

DISCUSSION

Recently, there have been increasing concerns about the effects of sweeteners on different physiological systems of the body (Ghusn *et al.*, 2023; Prada *et al.*, 2022; Singh *et al.*, 2023). The tendency to consume non-nutritive sweeteners for the sweet sensation they add to the diet, without adding

extra calories has increased (Faruque *et al.*, 2019). This study examines and compares the effects of consuming nutritive (sucrose) and nonnutritive (saccharin) sweeteners on male reproductive functions.

Sucrose, a nutritive sweetener, is derived primarily from the juice of *Saccharum officinarum* (sugarcane) and the roots of *Beta vulgaris* (sugar beet) (Gruska *et al.*, 2022), whereas saccharin, a nonnutritive sweetener, is manufactured from toluene or anthranilic acid (Azeez *et al.*, 2019).

Testosterone plays a crucial role in primary sexual development, which encompasses testicular descent, spermatogenesis, enlargement of the penis and testes, and increased libido (Kalfa *et al.*, 2019). In this study, in comparison with the control group, the sucrose group exhibited a significant reduction in testosterone levels, which is consistent with findings from Ashby *et al.* (2003) and Gong *et al.* (2016). Excessive sucrose consumption has been linked to apoptosis through overactivation of the testicular apoptotic pathway, leading to decreased testosterone levels (Glendinning *et al.*, 2015).

Luteinizing hormone (LH) is a glycoprotein hormone that is co-secreted with follicle-stimulating hormone (FSH) by gonadotrophin cells in the anterior pituitary gland (Kazmi and Can 2020). The results indicated that both the FSH and LH levels were lower in the sucrose group than in the control group. The cause of this decrease is unclear, as this finding opposes the basic physiological knowledge that expects a negative feedback-induced increase in gonadotropins stimulated by a decrease in testosterone (Marques *et al.*, 2000). Additionally, when the saccharin group was compared with the control group, the levels of luteinizing hormone, follicle-stimulating hormone, and testosterone also decreased. This finding suggests that there could be an alteration in the normal hypothalamo-pituitary-gonadal pathway resulting from excessive consumption of both natural and artificial sweeteners. This is in line with the reports of Puica and colleagues in 2009, that aspartame, an artificial sweetener, has a harmful effect on the hypothalamic-pituitary-gonadal axis (Puica *et al.*, 2009). In the study of male infertility, malondialdehyde (MDA) serves as a stable byproduct of polyunsaturated fatty acid

peroxidation and is often associated with protein crosslinking (Aitken *et al.*, 1987). Superoxide dismutase (SOD) is the primary detoxifying enzyme and a potent antioxidant within cells, playing a vital role as part of the first line of defense against reactive oxygen species (ROS) (Ighodaro and Akinloye, 2017). Catalase is another key antioxidant enzyme found in nearly all oxygen-utilizing tissues (Ighodaro and Akinloye, 2017). The results showed an increase in the MDA levels in both the sucrose and saccharin groups compared with those in the control group, with the MDA levels being significantly greater in the sucrose group (Adekunbi *et al.*, 2016). Elevated lipid peroxidation in the testes due to high sucrose levels may suggest free radical damage, further confirming its detrimental effects on reproductive function (Adekunbi *et al.*, 2016). Additionally, noncaloric sweeteners, such as artificial sweeteners, have been reported to increase MDA levels in the testes (Adekunbi *et al.*, 2016).

When the sucrose and saccharin groups were compared with the control group, a noticeable decrease in SOD activity was detected. Antioxidant enzymes are typically mobilized in response to rising free radical levels; however, when overwhelmed by excessive free radicals, oxidative stress can occur (Adekunbi *et al.*, 2016). Consequently, excessive sucrose consumption may induce oxidative stress in the testes, potentially impairing reproductive performance (Adekunbi *et al.*, 2016).

Compared with those in the control group, both the sucrose and saccharin groups exhibited lower catalase activity. Report has shown that administration of sucrose and saccharin led to a reduction in catalase activity (Husni *et al.*, 2023). There was a positive relationship between oxidative stress and saccharin consumption; saccharin could release ROS that might be counteracted by the body's ability to produce antioxidants such as catalase, which protects against ROS. This homeostatic mechanism involves a complex antioxidant defense system comprising several enzymes. However, if saccharin consumption continues at elevated doses, leading to increased free radical production, saccharin consumption could overwhelm antioxidant defenses and result in decreased catalase activity (Lobo *et al.*, 2010).

Sperm function—reflected by sperm count, motility, viability, and morphology—is a crucial indicator of male fertility potential (Moretti *et al.*, 2022). Compared with the control group, the sucrose group showed reductions in sperm count, motility, and viability. High sucrose levels have been shown to significantly disrupt sperm motility (Chiu *et al.*, 2014), with lower sperm motility and counts observed in young healthy men who consume high amounts of sugar-sweetened beverages (Chiu *et al.*, 2014). Furthermore, Gong *et al.* (2016) reported decreased sperm viability in the sucrose group alongside reductions in motility, characterized by fewer rapid sperm and a greater proportion of nonprogressive sperm. Compared with the control group, the saccharin group also presented decreased sperm count, motility, and viability. These findings align with those of Burke and Small (2015), who reported that high saccharin intake is associated with reduced sperm count and motility. Another study revealed that the total number of sperm significantly decreased in the high-dose saccharin group compared with the control group, while a

reduction in rapid sperm and an increase in immotile sperm were noted (Gong *et al.*, 2016).

Steroidogenic Acute Regulatory Protein (StAR) is essential for transporting cholesterol into mitochondria for steroid hormone synthesis, including testosterone (Stocco, 2001). The results indicated that StAR gene expression was lower in the sucrose group than in the control group; this finding is consistent with Gong *et al.* (2016), who reported a significant reduction in StAR expression in this group. A similar decrease was observed in the saccharin group relative to the control group. According to Gong *et al.* (2016), the saccharin group also presented lower StAR expression. This decline may correlate with increased cleaved caspase-3 levels—enzymes involved in apoptosis—suggesting potential damage to testicular function (Gong *et al.*, 2016).

In the histological examination of the testes, the control group (Figure 8) displayed tubules lined with a normal spermatogenic series (SS), Sertoli cells (SCs), and interstitial Leydig cells (LCs), which are essential for testosterone production in males. In contrast, the sucrose group exhibited degeneration of Leydig cells (LD) and tubular lumens filled with immature forms (IF), as depicted on figure 9. This degeneration may be attributed to sucrose exposure, which can induce oxidative stress. Oxidative stress damages Leydig cells, resulting in their degeneration and a subsequent decline in testosterone production (Aitken and Roman, 2008). Diets high in sugar have been linked to oxidative stress and impaired testicular function, with histological evidence showing disrupted spermatogenesis and the presence of immature germ cells within the tubular lumen (Lei *et al.*, 2001).

The saccharin group demonstrated patchy spermatogenic arrest (SA) and Leydig cell hyperplasia (LH), as illustrated in figure 10. This condition may also stem from oxidative stress, resulting in damage to sperm-producing cells (spermatocytes and spermatids), disrupting their development and leading to areas of arrested spermatogenesis (Monageng *et al.*, 2023). Elevated reactive oxygen species (ROS) levels can promote Leydig cell hyperplasia by altering the hormonal balance within the testes (Monageng *et al.*, 2023).

In the histology of the epididymis, the control group exhibited normal ducts (DU) filled with spermatozoa (SZ) and interstitial space (IS). The sucrose group showed significant depletion of spermatozoa in the ducts (SZ), as shown on figure 12. This depletion may be due to oxidative stress, as sucrose metabolism can generate reactive oxygen species (ROS), leading to oxidative damage. The epididymis is particularly susceptible to oxidative injury due to its high content of polyunsaturated fatty acids and low antioxidant capacity (Kumair and Sait, 2011). Additionally, sucrose intake has been associated with structural changes in the epithelial cells lining the epididymal ducts, including loss of cilia and degenerative alterations (Marcondes, 2018). The saccharin group also exhibited patchy ductal depletion of spermatozoa (SZ), as illustrated on Figure 13, likely due to oxidative stress. Saccharin may compromise antioxidant defense mechanisms in the epididymis, exacerbating oxidative stress and its detrimental effects on epididymal function (Vingren and Kraemer, 2010). Studies have shown that saccharin exposure in animal models leads to elevated levels of ROS and markers of oxidative damage in the

epididymis, which is correlated with the observed histological changes (Lei *et al.*, 2001).

In conclusion, both sucrose and saccharin adversely affect reproductive function primarily through mechanisms involving oxidative stress and the inhibition of steroidogenic acute regulatory protein (StAR), resulting in testicular damage, although sucrose had more negative effect when compared to saccharin.

REFERENCES

- Adekunbi, D.A., Ogunsola, O.A., Oyelowo, O.T., Aluko, E.O., Popoola, A.A. and Akinboboye, O.O. (2016). Consumption of high sucrose and/or high salt diet alters sperm function in male Sprague–Dawley rats. *Egyptian Journal of Basic and Applied Sciences*; 3:194-201.
- Aitken, R., Clarkson, J., Hargreave, T., Irvine, D. and Wu F. (1989). Analysis of the relationship between defective sperm function and the generation of reactive species in case of Oligozoospermia. *Journal of Andrology*; 10(3):214-220
- Aitken, R.J. and Roman, S.D. (2008). Antioxidant systems and oxidative stress in the testes. *Oxidative Med Cell Longev*; 1(1):15–24.
- Akhter, M., Biswas, P., Shahnaz, M., Rafiq, K. and Ali, T. (2023). Nigella Sativa oil on chronic constrictive injury (CCI) induced neuropathic pain of sciatic nerve in Wistar rats. *J Bangladesh Soc Physiol*; 18(1):35-45
- Akpantah, A.O., Oremosu, A.A., Ajala, M.O., Noronha, C.C. and Okanlawon, A.O. (2003). The effect of crude extract of Garcinia kola seed on the histology and hormonal milieu of male Sprague-Dawley rats' reproductive organs. *Nig. J. Health and Biomed. Sci*;2(1):40-46
- American Dietetic Association (ADA). (2004). Position of the American Dietetic Association: Use of nutritive and non-nutritive sweeteners. *J Am Diet Assoc* 2004; 104:255-275.
- Arshad, S., Rehman, T., Saif, S., Rajoka, M.S.R., Ranjha, M.M.A.N., Hassoun, A., Cropotova, j., Trif, M., Younas, A., Aadil, R. (2022). Replacement of refined sugar by natural sweeteners: focus on potential health benefits. *Heliyon* ; 8(9):e10711
- Ashby, J., Tinwell, H., Lefevre, P.A., Joiner, R. and Haseman J. (2003). The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisfenol A between postnatal days 91-97. *Toxicology science*; 74:129-138.
- Azeez, O.H., Alkass, S.Y. and Persike, D.S. (2019). Long-term saccharin consumption and increased risk of obesity, diabetes, hepatic dysfunction, and renal impairment in rats. *Medicina (Kaunas)*; 55(10):681
- Burke, M.V. and Small, D.M. (2015). Physiological mechanisms by which non- nutritive sweeteners may impact body weight and metabolism. *Physiology Behaviour* 2015; 152:381-388.
- Chiu, Y.H., Afeiche, M.C., Gaskins, A.J., Williams, P.L., Mendiola, J., Jorfensen, N., Swan, S.H. and Chavarro, J.E. (2014). Sugar-sweetened beverage intake in relation to semen quality and reproductive hormone levels in young men. *Hum Reprod.*; 29(7)1575-1584.
- Elekofehinti, O.O. Onunkun, A.T. Olaleye, M.T. (2020). Cymbopogon citratus (DC.) Stapf mitigates ER-stress induced by streptozotocin in rats via down-regulation of GRP78 and up-regulation of Nrf2 signaling *Journal of Ethnopharmacology*; 262: 113130.
- Faruque, S., Tong, J., Lacmanovic, V., Agbonghae, C., Minaya, D.M. and Czaja, K. (2019). The dose makes the poison: sugar and obesity in the United States – a review. *Pol J Food Nutr Sci*; 69(3):219-233.
- Fitch, C., Keim, K.S. (2012). Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. *J Acad Nutr Diet*; 112(5):739-758
- Ghush, W., Naik, R. and Yibirin, M. (2023). The impact of artificial sweeteners on human health and cancer association: A comprehensive clinical review. *Cureus*; 15(12):e51299
- Glendinning, J.I., Stano, S., Holter, M.A.T., Goldman, O., Margolskee, R.F., Vasselli, J.R. and Sclafani, A. (2015). Sugar-induced cephalic-phase insulin release is mediated by a T1r2bT1r3-independent taste transduction pathway in mice. *Stat Pearls*; 309:552–560.
- Gong, T., Wei, Q., Mao, D. and Shi, F. (2016). Expression patterns of taste receptor type 1 subunit 3 and alpha-gustducin in the mouse testis during development. *Acta Histochemistry*; 118:20–30.
- Gruska, R.M., Baryga, A., Kunicka-Styczynska, A., Brzezinski, S., Rosicka-Kaczmarek, J., Miskiewicz, K. and Suminska, T. (2022). Fresh and stored sugar beet roots as a source of various types of mono- and oligosaccharides. *Molecules*; 27(16):5125
- Handelsman DJ. (1998). Pharmacokinetics and pharmacodynamics of testosterone pellets in man. *Journal of Steroid Biochemistry and Molecular Biology*; 65(1-6):61-66.
- Helal, E.G., Abdelaziz, M.A. and El-Gamal, M.S. (2019). Effects of süßstofftableten on some physiological parameters in male albino rats and the curative effects of vitamin E. *Egyptian Journal of Hospital Medicine*; 75(4).
- Howard, B.V. and Wylie-Rosett, J. (2002). Sugar and cardiovascular disease. *Circulation*; 106:523-535.
- Husni, M.H., Suad, Y.A. and Daniele, S.P.O. (2023). Impact of long-term cyclamate and saccharin consumption on biochemical parameters in healthy individuals and type 2 diabetes mellitus patients. *Medicina*; 59:698.
- Ighodaro, O.M. and Akinloye, O.A. (2017). First line defence antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*; 54:87-293.
- Kalfa, N., Gaspari, L.O.M., Bergougnoux, P.P.A. and Sultan, P.F.C. (2019). Molecular genetics of hypospadias and cryptorchidism recent developments. *Clin Genet*; 95(1):122-131.
- Kazmi, S.R.H. and Can A.S. (2020). Luteinizing Hormone Deficiency. (FL): *Europe PMC*; PMID:32965890.
- Kumari, S. and Sait, S.F. (2011). Role of steroidogenic acute regulatory protein in the mechanism of action of endocrine disruptors. *Journal of Steroid Biochemistry and Molecular Biology*; 127(3-5):187-194.
- Lei, Z.M., Mishra, S., Zou, W., Xu, B., Foltz, M., Li, X. and Rao CV. (2001). Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Molecular Endocrinology*; 15(1):84-200.
- Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*; 4(8):118-126
- Marcondes, R.R. (2018). Impact of endocrine disruptors on reproductive health. *Journal of Endocrinology and Metabolism*; 26(4):345-359.
- Marques, P., Lages, A.D.S., Skorupskaitė, K., Rozario, K.S., Anderson, R.A. and George, J.T. (2000). Physiology of GnRH and gonadotropin secretion. Edotext [Internet], MD Text.com, Inc; PMID:25905297.
- Misra, H.P. and Fridovich I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay

- for superoxide dismutase. *J. Biol. Chem*; 247(10):3170-3175.
- Mongeng, E., Offor, U., Takalani, N.B., Mohlala, K. and Opuwari, C.S. (2023). A review on the impact of oxidative stress and medicinal plants on Leydig cells. *Antioxidants*; 12(8):1559
- Moretti, E., Signorini, C., Noto, D., Corsaro, R. and Collodel, G. (2022). The relevance of sperm morphology in male infertility. *Front Reprod Health*; 4:945351
- Okeke, O.C., Ekakitie, O.O., Adeniyi, M.J., Oyeyemi, A.W. and Ajayi, O.I. (2023). Interrelationship between surging reproductive hormones and blood viscosity indices in apparently healthy females. *JKIMSU*; 12(2):57-64
- Olumegbon, L.T., Lawal, A.O., Oluyede, D.M., Adebimpe, M.O., Elekofehinti, O.O, Umar, H.I (2022). Hesperetin protects against diesel exhaust particles induced cardiovascular oxidative stress and inflammation in Wistar rats. *Environmental Science and Pollution Research*, 29(35):52574-52589
- Oyeyemi, W.A., Daramola, O.O., Akinola, A.O., Idris, A.O., Aikpitanyi, I. (2020): Hepatic and reproductive toxicity of sub-chronic exposure to dichlorvos and lead acetate on male Wistar rats. *Asian Pac. J. Reprod.*; 9(6):283-290
- Prada, M., Saraiva, M., Garrido, M.V., Serio, A., Teixeira, A., Lopes, D., Silva, D.A. and Rodrigues, D.L. (2022). Perceived association between excessive sugar intake and health conditions. *Nutrients* 2022; 14(3):640
- Puica, C., Craciun, C., Rusu, M., Cristescu, M., Borsa, M. and Roman I. (2009). Ultrastructural aspects concerning the hypothalamus-pituitary complex reactivity following chronic administration of aspartame in juvenile rats. *Studia Universitatis Vasile Goldis Arad, Seria Stiintele Vietii*; 19(1):19-24.
- Reed, D.N., McDaniel, A.H. (2006). The Human Sweet Tooth. *BMC Oral Health*; 16(1):17
- Singh, S., Kohli, A., Triverdi, S., Kanagala, S.G., Anamika, F.N.U., Garg, N., Patel, M.A., Munjal, R.S., Jain, R. (2023). The contentious relationship between artificial sweeteners and cardiovascular health. *The Egyptian Journal of Internal Medicine*; 35:43
- Stocco, D.M. and Clark, B.J. (1996). Regulation of the acute production of steroids in steroidogenic cells. *Endocrine Reviews*; 17(3):221-244.
- Tannenbaum, L.V., Bazar, M., Hawkins, M., Cornaby, B.W., Ferguson, E.A., Carroll, L.C. and Ryan, P.F. (2003). Rodent Sperm Analysis in Field-Based Ecological Risk Assessment: Pilot Study at Ravenna Army Ammunition Plant, Ravenna, Ohio. *Environmental Pollution*; 123(1):21-29.
- Tiwana, M.S. and Leslie S.W. (2017). Anatomy, Abdomen and Pelvis: Testes. *StatPearls*. PMID:29261881
- Vingren, J.L. and Kraemer, W.J. (2010). Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. *Sports Medicine*; 40(12):1037-1053.

Full length Research Article

Bromelain Improved Cognitive & Mood Behaviors, Oxidative-inflammatory Indices and Cholinergic Transmission in Scopolamine-Induced Neurotoxicity in Male Wistar Rats.

Bayo-Olugbami A.A.¹, Ukpabio P.², Babalola K.M.³, Benson I.O.⁴, Owoyele B.V.⁵

¹Neuroscience unit, Department of Physiology, Osun State University, Osogbo. Osogbo, Nigeria

²Department of Physiology, Adeleke University, Ede. Osun State, Nigeria

³Department of Anatomy, Federal University of Health Sciences, Ila-Orangun. Osun State, Nigeria

⁴Department of Anatomy, Osun State University, Osogbo. Osun State, Nigeria

⁵Neuroscience & Inflammation unit, Department of Physiology, University of Ilorin, Ilorin, Nigeria

Summary: Alzheimer's disease (AD) is associated with pathophysiological and psychological disturbances including cognitive decline, depression, anxiety and motor imbalance. Conventional drugs for managing AD do not address associated non-cognitive co-morbidities. Hence, the need to investigate alternative therapies especially from plants. The neuroprotective benefits of bromelain have been identified, but its impacts on scopolamine-induced neurotoxicity is yet to be elucidated. Twenty-Five male rats were separated randomly into 5 groups: Control (normal saline; 1ml/kg); Scopolamine (i.p; 1mg/kg); Bromelain (50mg/kg); Scopolamine + Bromelain; Scopolamine + Donepezil (reference drug, 1mg/kg). Neurobehavioral paradigms (novel object recognition, elevated plus maze, forced swimming and open field tests) were assessed, followed by biochemical assay of Malondialdehyde, MDA; Super oxide dismutase, SOD; Acetylcholinesterase, AChE; Nitric oxide, NO; Total protein & Interleukin 1 β , IL-1 β in the Prefrontal cortex (PFC). Data were analyzed using one-way ANOVA (Tukey's posthoc.). Values with $p < 0.05$ were considered significant. Scopolamine reduced memory index ($P < 0.01$) (cognitive function), ambulatory & rearing activities ($P < 0.05$) (motor behavior), open arm duration ($P < 0.001$) (anxiety-like behavior) & increased immobility time ($P < 0.05$) (depressive-like behavior). Exposure to scopolamine also led to significant reduction in the prefrontal cortical level of SOD ($P < 0.05$) while increasing MDA ($P < 0.05$), acetylcholinesterase ($P < 0.01$) and IL-1 β ($P < 0.05$). However, levels of NO and total protein were not significantly altered. In contrast, intervention with bromelain or donepezil significantly reversed most of the behavioral and biochemical alterations induced by scopolamine. Bromelain compared favorably with donepezil in improving memory decline and other non-cognitive dysfunctions associated with scopolamine exposure. This could have resulted from modulation of oxidative stress, inflammation and cholinergic transmission.

Keywords: Bromelain; Scopolamine; Oxidative stress; Neurotoxicity; Cognition; Mood.

*Authors for correspondence: adedamola.bayo-olugbami@uniosun.edu.ng, Tel: +234-8132794032

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INTRODUCTION

Cognitive dysfunction which characterizes Alzheimer's disease (AD) is caused by multiple factors including deposition of amyloid plaques, oxidative stress, neuro-inflammation and cholinergic dysfunction (Ayinla *et al.*, 2020). Apart from cognitive decline, AD is associated with pathophysiological and psychological disturbances including motor imbalance, depression and anxiety, usually referred to as behavioral and psychological symptoms of dementia (BPSD) (Brechtin *et al.*, 2013). Up to 75% of patients with dementia experience these non-cognitive symptoms, which provide a significant challenge for patients and their carers, and are often difficult to manage. Conventional drugs for managing dementia do not address associated non-cognitive co-morbidities. Antipsychotic

drugs have been widely used to treat non-cognitive symptoms of dementia. However, data emerged linking the use of some antipsychotic drugs with an increased risk of stroke and death in patients with dementia (Kales *et al.*, 2012). Consequently, there has been a world-wide drive to seek appropriate non-pharmacologic interventions like diet modification and exercise (Livingston *et al.*, 2005; Fernando *et al.* 2015). Hence the need to investigate alternative therapies especially from plants that can provide holistic protection or treatment.

Scopolamine is a non-selective post-synaptic muscarinic receptor blocker. It causes cognitive impairments in rodents and humans by decreasing the effectiveness of acetylcholine in the CNS (Kim *et al.*, 2013). Though scopolamine is not a classical model for inducing experimental AD in preclinical

models, however, it presents one of the best approaches for understanding the mechanism of AD and other types of dementia; and also widely used as a valid pharmacological model for investigating and screening potential anti-amnesic molecules (Blokland, 2005; Haider *et al.*, 2016).

The use of natural products in the prevention and treatment of diseases have gained significant attention. Bromelain is a group of sulfur-containing proteolytic enzymes present in pineapple (*Ananas comosus*), a tropical plant widely cultivated worldwide (Chaisakdanugull *et al.*, 2007). It is made up of several proteinases, flavonoids and endopeptidases (Tochi *et al.*, 2008) with many uses as natural anti-inflammatory, anti-oxidant, antithrombotic, and fibrinolytic agent. Because of its history of safety and little to no side effects, it has gained universal acceptance as a phytotherapeutic agent (Chakraborty *et.*, 2021).

Bromelain reportedly reduced MDA and increased the concentrations of SOD, CAT, GPx in a rat model of bisphenol A-induced oxidative stress and testicular damage. Bromelain has antioxidant activities through free radical scavenging and up-regulation of enzymatic and non-enzymatic antioxidants (Agarwal *et al.*, 2016). Another study demonstrated the beneficial effects of bromelain in reducing neurotoxicity through antioxidant and AChE-inhibitory effects (Bist *et al.*, 2021). Bromelain elicits anti-inflammatory response by reducing PGE2 and COX-2 synthesis (Bhui *et al.*, 2009). It acts as an antioxidant by stimulating antioxidant enzyme secretion of catalase, superoxide dismutase, and reduced glutathione via increased concentration of nuclear factors (NrF-1 and NrF-2) (Bakare & Owoyele, 2020). Many pharmacological benefits of bromelain have been identified, but its impacts on scopolamine-induced neurotoxicity is yet to be elucidated.

In a rat model of AlCl₃-induced toxicity, treatment with bromelain ameliorated the effects of AlCl₃ by restoring locomotion and exploratory activity, improving cognitive functions, and inducing anxiolytic effects. Further, the effects of bromelain were comparable to donepezil therapy (Eraky *et al.*, 2023). Similarly, it was shown to reduce anxiety-like behavior in a rat model of neuropathic pain (NP) where it decreased the cortical levels of IL-1 β , IL-6 and PGE2. It also reduced NF- κ B, IL-1 β , IL-6, TNF- α , PGE2, and nitrate concentrations as well as the expression of iNOS in the sciatic nerve. Hence, the anti-nociceptive and anxiolytic effects of bromelain in the sciatic nerve ligation model of NP was ascribed to its ability to reduce nitrosative and inflammatory activities (Bakare & Owoyele, 2021).

In this study, we investigated if scopolamine mechanistic model of studying dementia could show non-cognitive (anxiety- and depressive like) symptoms of dementia in addition to the classical cognitive impairment; and to determine the neuroprotective impact(s) of bromelain in comparison to donepezil (a reference drug) on both cognitive and non-cognitive symptoms that may be associated with scopolamine-induced neurotoxicity and its mechanism(s) of action.

MATERIALS AND METHODS

Experimental Animals: Twenty-five adult male rats with an average weight of 115 ± 5.1 g were obtained from the central animal house, University of Ibadan. The rats were

kept in the animal facility of the Faculty of Basic Medical Sciences, Adeleke University, Ede, Nigeria. They were kept in polypropylene plastic cages with wood shavings as bedding at a room temperature of (27-30°C) with 12 h light/dark cycle. Rats had access to standard rodent pellet food and water ad libitum. Acclimatization was for 7 days. Approval for this study was provided by the Adeleke University Animal Care and Use Ethical Review Committee (Ethical approval number: AUERC/FBMS/20) which complies with the National Institutes of Health (NIH) Guideline for the Care and Use of Laboratory Animals.

Drug/chemical preparations and dosages: The drugs and chemicals used were of analytical grade. Scopolamine and donepezil were products of Macleods Pharma (Billingham, UK). Bromelain powder was produced by KAN Phytochemicals (Rai, India). The doses of bromelain (50 mg/kg) (Bakare & Owoyele, 2020; Bakare & Owoyele, 2021), scopolamine (1mg/kg) (Ayinla *et al.*, 2019; Ayinla *et al.*, 2020; Oladun *et al.*, 2023), donepezil (1mg/kg) (Filarowska *et al.*, 2016; Eraky *et al.*, 2023) were selected according to findings from preliminary and previous reports. Saline (0.9%) was used to dissolve bromelain, scopolamine and donepezil and were administered at a volume of 5ml/kg of individual animal weight.

Experimental design: Twenty-five male Wistar rats were divided into five groups. Group one (control) received 5ml/kg of normal saline. Group 2 received 1mg/kg of scopolamine. Group 3 received 50mg/kg of Bromelain. Group 4 received both scopolamine and Bromelain. Group 5 received scopolamine and donepezil (Reference drug). The experiment lasted for 15 days. Bromelain or donepezil was administered for 14 days, with concurrent administration of scopolamine in the last 7 days. During co-administration, bromelain or donepezil was given 30 minutes before scopolamine. Behavioral assessments for motor function using horizontal bar test and open field test; cognitive function using novel object recognition test; anxiety using elevated plus maze test and open field test; and depression using forced swimming test were carried out on the last two days of the experiment. Rats were sacrificed on day 15 for biochemical processing. Cortical assessment of SOD, MDA, NO, AChE, total protein and IL-1b were quantified spectrophotometrically.

Animal grouping:

Control group: Normal saline at 5ml/kg orally for 14 days

Scopolamine group: 1mg/kg of Scopolamine (i.p.) in the last 7 days (Mostafa *et al.*, 2021; Bayo-Olugbami *et al.*, 2024).

Bromelain group: 50mg/kg of Bromelain orally for 14 days.

Bromelain + Scopolamine group: 50mg/kg of Bromelain (oral) for (14) days + concurrent administration of 1mg/kg of Scopolamine (i.p.) in the last 7 days

Scopolamine + Donepezil group: 1mg/kg of Donepezil (oral) for 14 days + concurrent administration of 1mg/kg of Scopolamine (i.p.) in the last 7 days.

Behavioral Assessment: Behavioral tests were carried out on the last two days of the experiment. Animals were evaluated for behavioral phenotypes in the following order: (a) open field & horizontal bar tests, for motor or exploratory assessment (b) novel object recognition test for assessment of cognitive function (c) elevated plus maze test for assessment of anxiety-like behavior and (d) forced swimming test for assessment of depressive-like behavior. All behavioral tests were carried out between 9:00 a.m.-1:00 p.m. each day by trained observers who were blind to the treatment groups.

Open field test: This was used to assess locomotion and exploratory activities of rats. An open wooden box, measuring 100 x 100 x 50 cm was used. The rat was placed at the centre square. A high resolution video recorder (Logitech C920 HD) kept at a safe distance to cover the entire field was used in recording the activities of each rat in the box for a duration of 6 minutes. The number of lines crossed (horizontal exploration), rearing (vertical exploration) and frequency of centre exploration were later scored by an independent investigator. The number of visit to the centre of the open field maze and fecal boli voided are another good indicator of anxiety-like behavior. This is done by estimating the frequency of visit to the central zone of the open field maze and the number of fecal pellets voided in the process of exploring the maze within 6mins. An increased central visit or reduced peripheral/wall zone exploration (thigmotaxis) and decreased fecal output are indicative of anxiolytic (reduced anxiety) propensity. Olfactory cue was reduced by removing rat's litters and cleaning the test field with 70% ethanol after each experiment.

Horizontal bar test: A horizontal bar 38 cm long, 3 cm in diameter, and 50 cm above the bench surface was used in a modified technique (Deacon, 2013), with a wooden support column at each end. Each rat was grasped by the tail, slanted backwards about 20 cm, and immediately raised to hold the centre point of the horizontal bar with its forepaws while the tail was released. The time taken by each rat to move through the bar was recorded in seconds.

Novel Object recognition test

Novel Object Recognition (NOR): This test assesses non-spatial short term memory in animals as originally described by Bevins and Besheer, (2006) but with slight modification by Bayo-Olugbami *et al.* (2020). These tests rely on the fact that rodents generally will preferentially explore a novel object over a previously experienced object. A 45cm by 50cm opaque box was used. Rats were allowed to explore two identical objects placed 5cm apart from each other and from the walls of the box for 10 minutes (training section) in trial 1 (T1). An inter trial period of about 30 minutes was allowed after which each rat was returned to the test field for another 6 minutes in T2, during which one of the old objects was replaced with a new object (test session). Exploration was scored positive when the nose or vibrissae is about 2cm from the object while sitting on the object was excluded. A rat not used in the experiment was allowed to explore the arena prior to acclimatization, training, and testing so that the field would have a familiar odor to the first rat to be tested each day. The placement of the objects

was changed frequently while the box arena and objects used were thoroughly wiped with 70% ethanol after each test before introducing the next rat in order to reduce olfactory cues. The session was video recorded and later evaluated. The memory index was calculated as: (Time spent exploring new object/ total time spent exploring both objects) $\times$ 100

Elevated plus maze test: Elevated plus maze test (EPMT) is commonly used to assess anxiety-like behavior as previously described by Lister (1987). The rats were individually placed in the central zone facing one of the open arms. Video camera mounted above the maze connected to a computer was used to monitor and score the exploratory behavior during 5mins experimental period. The parameter measured was the duration of open arm entry. An entry was scored when the four paws of the animals were completely inside any of the arms. An increased open arm entry or exploration reflects a lesser tendency for anxiety behavior.

Forced swimming test: It was performed as originally described by Porsolt and co-workers (Porsolt *et al.*, 1978). The apparatus consists of a clear plexiglass cylinder (20cm by 12cm) filled to a 15cm depth with water. Rats were pre-exposed to swimming environment for five minutes a day prior to the test. Thirty minutes after treatment, animals were gently placed inside the cylinder and allowed to swim for 6 minutes. Duration of Immobility was assessed during the last five minutes using an automated stopwatch. The rats were assumed immobile when they floated and made only movements necessary to keep their heads above water. Higher immobility time indicates depressive-like behavior (Yankelevitch-Yahav *et al.*, 2015).

Animal sacrifice, sample collection and preparation of tissue for biochemical assays: After behavioral evaluations, rats were anaesthetized with ketamine (50mg/kg)/Xylazine (10mg/kg) cocktail, subjected to trans-cardiac perfusion using 50ml 0.1 M PBS (pH 7.4). Rat was decapitated and the excised brain (PFC) was rinsed in cold isotonic saline. PFC was isolated and homogenized over ice in 0.1 M cold sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm, 4°C for 10 min. The supernatant obtained was made into aliquots for determination of markers of oxidative stress, inflammation, and acetylcholinesterase activity.

Determination of oxidative stress markers: The superoxide dismutase (SOD): SOD activity was determined according to the method of Sun *et al.* (1988). The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate after 1.0 ml ethanol-chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate.

The Malondialdehyde (MDA): MDA, one of the by-products of peroxidation of polyunsaturated fatty acid was used to determine the oxidative stress levels in the prefrontal

cortex. This assay was performed according to the method of Ohkawa *et al.* (1979) in which MDA reacted with Thiobarbituric acid (TBA) in an acidic medium at 100°C to produce a pink/red-coloured product (Thiobarbituric acid reactive substances) extracted with butanol and measured using a spectrophotometer at an absorbance of 520-535nm.

NO: Nitric oxide was assayed according to a previous method by Tracey *et al.* (1995) using Griess reagent system with a few modifications (Sun *et al.*, 2003). 0.1% w/v NED solution (naphthyl ethylene diamine di-hydrochloride) was used. The reaction mixture containing 50 µl supernatant and 50µl PBS were incubated at 25°C for 15 min. Then 50 µl of sulphanilamide solution (1% sulphanilamide in 5% phosphoric acid) was added. After 5 min, the absorbance was measured at a wavelength of 540 nm against the corresponding blank solutions. Sodium nitrite was used as a standard sample.

Total protein: The total protein was determined according to the method of Bradford (1976) using Pierce Coomassie Protein assay kit, a commercially ready to use kit procured from Sigma, USA. When mixed with the protein solution, the acidic Coomassie –dye reagent changed color from brown to blue in proportion to the amount of protein present in the sample. Absorbance was read with a spectrophotometer at 595nm.

Determination of cholinergic marker: Estimation of acetyl-cholinesterase activity: AChE was quantified in the prefrontal cortex using the Ellman method (Ellman *et al.*, 1961). In this test, the Ellman's reagent, 5, 5'- dithiobis (2-nitrobenzoate), (DTNB) was used to assay for free thiol groups. The phosphate buffer (2.7 mL) and 0.1 mL of DTNB were added to the homogenate and allowed to stand for 5 min. Following the addition of 0.1 mL of newly made acetylthiocholine iodide (pH 8), the absorbance was read at 412 nm.

Determination of Inflammatory marker: The concentration of IL-1b in the PFC was determined using enzyme-linked immunosorbent assay kits (Nanjing Mornmed Medical, Nanjing City, Jiangsu province, China) following the manufacturers' guidelines. All the measurements were done at room temperature using microplate reader with 450 nm filter (Micro READ 1000, Belgium). The concentrations of IL-1b was extrapolated from the standard curve and expressed as pg/mg protein.

Statistical Analysis: GraphPad Prism version 8.0 was used for all statistical analyses. All data were expressed as Mean±SEM. Differences among the groups were analyzed by one-way ANOVA using Tukey's post hoc for multiple comparison. P value < 0.05 was considered to be statistically significant.

RESULTS

Effects of bromelain on behavioral assessment in scopolamine-induced neurotoxicity: We investigated the impacts of scopolamine on cognitive and non-cognitive comorbidities like motor, anxiety- and depressive-like

behaviors using appropriate protocols; and also determined the neuroprotective effects of bromelain on such deficits.

Effects of bromelain on locomotion and exploration in the open field test (OFT) following scopolamine exposure:

Number of lines crossed: As shown in Fig 1a, rats in Scopolamine (SCOP) group had significantly lower total number of line crossed while exploring the maze compared to control ($p < 0.05$ vs control group), indicating an impaired locomotor and exploratory activity. Number of lines crossed was markedly increased in Bromelain (BROM) only group compared with control ($p < 0.01$ vs control group). Conversely, only BROM group significantly increased number of lines crossed when compared to SCOP group ($p < 0.01$ vs SCOP group). SCOP+BROM group showed reduction in number of lines crossed compared with BROM only group ($p < 0.01$). Horizontal exploration was also reduced in the SCOP+DONE group compared with SCOP+BROM group ($p < 0.05$).

Rearing frequency: In Fig 1b, vertical exploration depicted by rearing frequency was significantly lower in the SCOP ($P < 0.001$) and SCOP+DONE ($p < 0.01$) groups when compared with control. However, BROM only ($p < 0.01$) and SCOP+BROM ($p < 0.05$) groups showed a marked increase in rearing frequency compared with SCOP group.

Time spent on bar: In Fig 1c, rats in SCOP group spent longer time moving through the bar compared with control ($p < 0.05$). However, the groups that received BROM only ($p < 0.01$), SCOP+BROM and SCOP+DONE ($p < 0.05$) spent lesser time on bar which implies improved motor activity.

Centre exploration: As shown in Fig 1d, the time spent at the centre of the maze (an indicator of anxiety) was significantly lower in the SCOP group compared with control ($p < 0.001$). In contrast, BROM only ($p < 0.001$), SCOP+BROM and SCOP+DONE ($p < 0.05$) showed marked increase in the number of times rats visited the centre of the maze.

Number of fecal boli: This is also used to estimate anxiety-like behavior. As shown in Fig 1e, rats in the SCOP group had significant increase in the number of fecal pellets when compared with control group ($p < 0.05$), implying anxiety-like propensity. Only BROM group markedly reduced the number of fecal pellets compared with SCOP group ($p < 0.05$).

Effects of bromelain on cognitive function in the novel object recognition test (NORT) following scopolamine exposure:

Fig 2 shows that exposure to SCOP caused a significant reduction in memory index compared with control ($p < 0.01$). This shows that the rats spent less time exploring the novel one, indicating a low novelty preference. Conversely, the groups that received BROM only ($p < 0.05$), SCOP+BROM ($p < 0.05$) and SCOP+DONE ($p < 0.001$) had increased memory index compared with SCOP only group which implies an improvement in memory function.

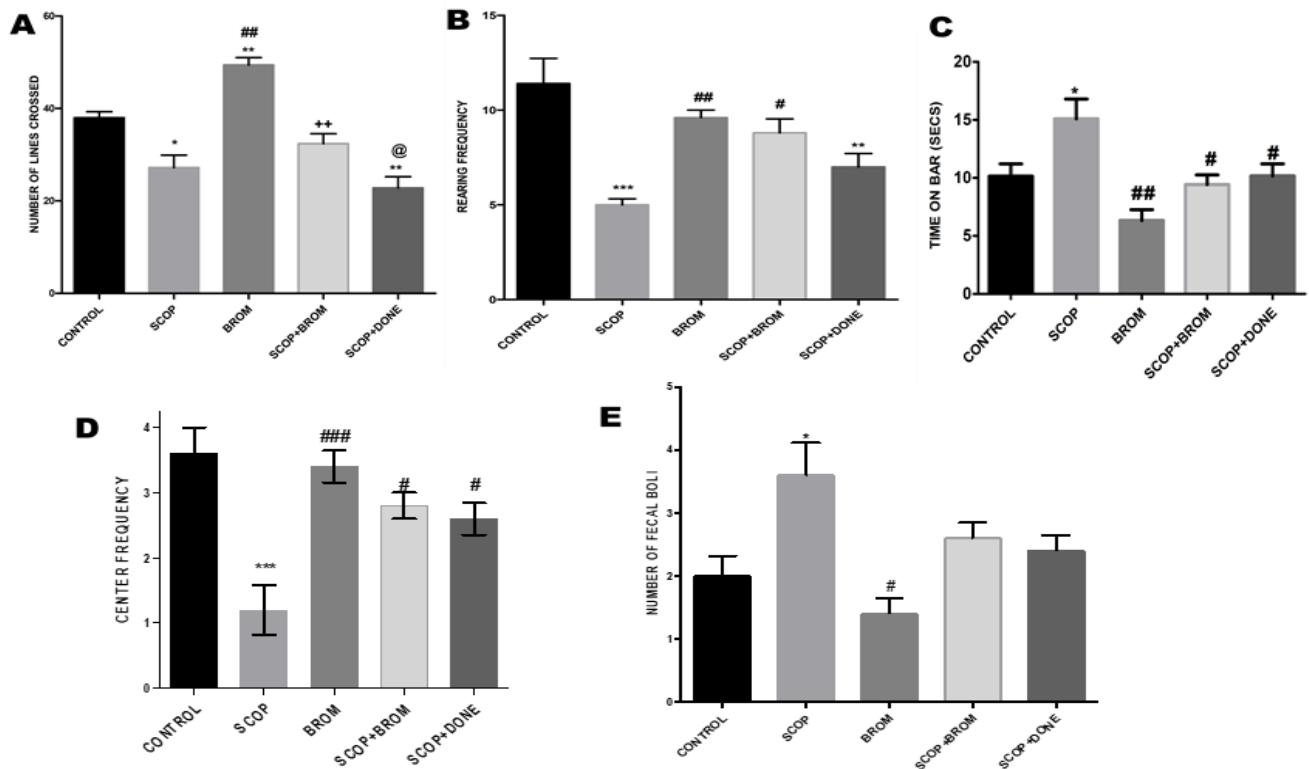


Fig 1: Effects of bromelain on the number of lines crossed (A), rearing frequency (B), time on bar (C), centre exploration (D) & number of fecal boli (E) in scopolamine induced neurotoxicity in rats.

Values are expressed as mean $\pm$ SEM (n=5).

*P<0.05 vs control, **P<0.01 vs control, ***P<0.001 vs control; #P<0.05 vs Scopolamine, ##P<0.01 vs Scopolamine, ###P<0.001 vs Scopolamine; @P<0.05 vs Scop+Brom, ++P<0.01 vs Bromelain. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil

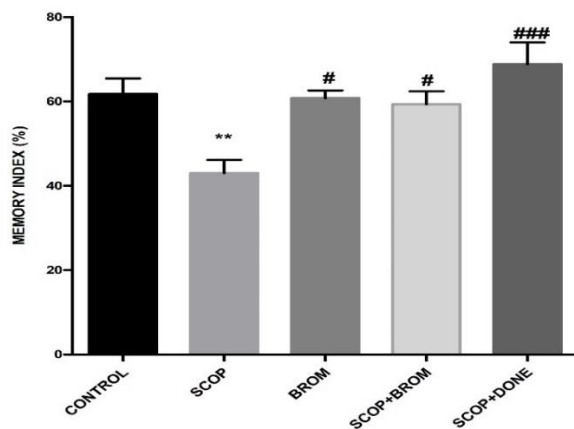


Figure 2:

Effects of bromelain on memory index in scopolamine induced neurotoxicity in rats.

Values are expressed as mean $\pm$ SEM (n=5).

**P<0.01 vs control; #P<0.05 vs Scopolamine, ###P<0.001 vs Scopolamine. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil

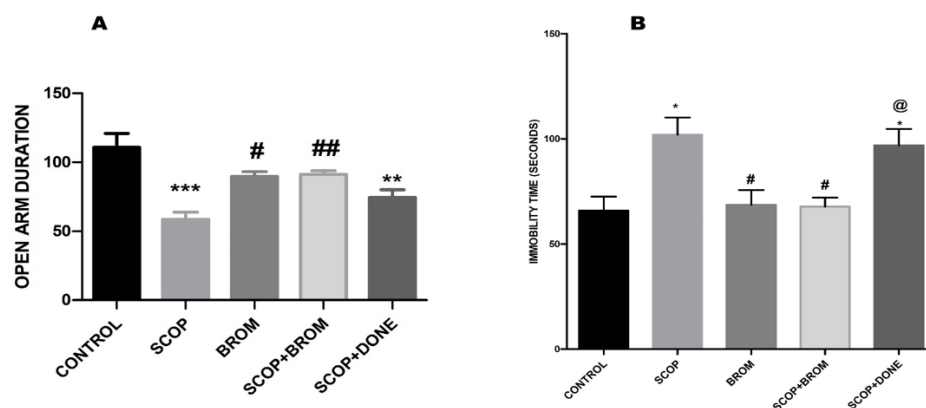


Fig 3: Effects of bromelain on open arm duration in EPM test (A) and immobility time in FST (B) in scopolamine-induced neurotoxicity in rats.

Values are expressed as mean $\pm$ SEM (n=5). *P<0.05 vs control, **P<0.01 vs control, ***P<0.001 vs control; #P<0.05 vs Scopolamine, ##P<0.01 vs Scopolamine; @P<0.05 vs Scop+Brom. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil, EPM: elevated plus maze, FST: forced swimming test.

Bromelain reduced scopolamine neurotoxicity

Effects of bromelain on mood (anxiety- and depression-like behavior) in the elevated plus maze and forced swimming test following scopolamine exposure: Time spent in the open arm: In Fig 3a, rats in the SCOP group ($p<0.001$) and SCOP+DONE ($p<0.01$) spent significantly lower time in the open arm of EPM compared with control rats. However, only BROM ($p<0.05$) and SCOP+BROM ($p<0.01$) groups showed significant increase in the time spent in open arm which indicates anxiolytic propensity. However, donepezil showed no anxiolytic property.

Immobility time: In Fig 3b, there was a significant increase in the immobility time in SCOP rats compared to control ($p<0.05$). This depicts depressive-like action. Administration of BROM only and SCOP+BROM ($p<0.05$) reduced the depressive-like symptom as depicted by a lower immobility time compared with SCOP untreated group. In addition, the immobility time in the SCOP+DONE group was also markedly higher compared to SCOP+BROM group ($p<0.05$). This shows that bromelain reduced depression but donepezil did not show anti-depressive propensity in scopolamine-induced neurotoxicity in rats.

Effects of bromelain on prefrontal cortical levels of oxidative stress, cholinergic and inflammatory markers following scopolamine exposure:

SOD: cortical SOD (an anti-oxidant enzyme) level was significantly lower in SCOP group compared to control ($p<0.05$). In contrast, rats in BROM, SCOP+BROM and SCOP+DONE ($p<0.01$) groups all had a marked increase in the level of SOD when compared with SCOP only, indicating an upregulation in the level of the anti-oxidant enzyme (Fig 4a).

MDA, a marker of lipid peroxidation was markedly reduced in the SCOP group ($p<0.05$) when compared with control.

The groups that received BROM ($p<0.01$), SCOP+BROM ($p<0.01$) and SCOP+DONE ($p<0.001$) had a marked reduction in the level of MDA when compared with SCOP (Fig 4b).

NO: In Fig 4c, nitric oxide, a marker of oxidative stress was empirically increased in the SCOP group but significantly lower in BROM only group ($p<0.05$) when compared to control. However, BROM ($p<0.01$) and SCOP+BROM ($p<0.05$) groups had a significant reduction in NO compared with SCOP untreated rats. In addition, SCOP+DONE group showed marked reduction in the level of NO compared to BROM only group ($p<0.05$).

Total protein: Fig 4d shows an insignificant reduction in the level of total protein in the SCOP exposed rats when compared with control group. Only BROM group was able to increase TP level significantly compared with untreated SCOP rats ($p<0.05$). Similarly, intervention with bromelain and donepezil following scopolamine exposure also showed empirical increase in the level of total protein.

AChE: Prefrontal cortex level of AChE was markedly increased in SCOP exposed rats compared with control ($p<0.01$). The groups that received BROM ($p<0.05$), SCOP+BROM and SCOP+DONE ($p<0.001$) had significantly lower level of AChE compared with SCOP rats. This might be interpreted to mean that cholinergic activity was preserved as a result of reduced acetylcholinesterase activities (Fig 4e).

IL-1 β : As shown in Fig 4f, the level of IL-1 β was markedly increased in SCOP rats compared with control ($p<0.05$). Both BROM ($p<0.01$) and SCOP+BROM ($p<0.05$) groups showed a marked decline in the level of IL-1 β when compared with SCOP only.

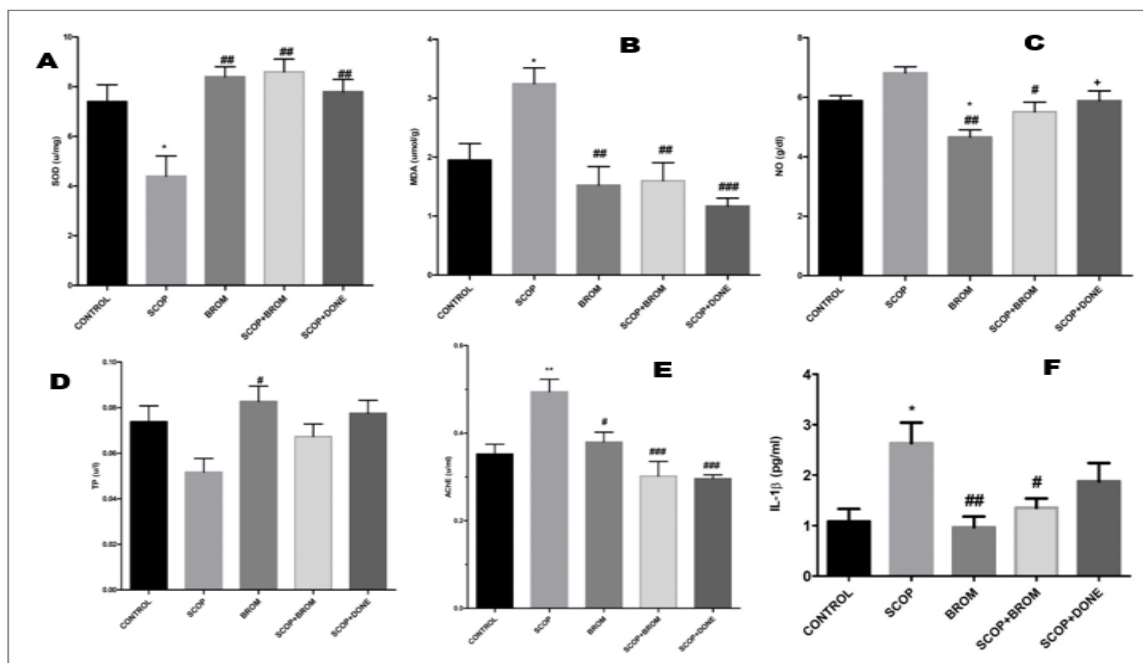


Figure 4:

Effects of bromelain on prefrontal cortical levels of SOD (A), MDA (B), NO (C), Total protein (D), AChE (E) & IL-1 β (F) in scopolamine induced neurotoxicity in rats.

Values are expressed as mean $\pm$ SEM (n=5).

* $P<0.05$ vs control, ** $P<0.01$ vs control; # $P<0.05$ vs Scopolamine, ## $P<0.01$ vs Scopolamine, ### $P<0.001$ vs Scopolamine; * $P<0.05$ vs Bromelain. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil, SOD: Superoxide dismutase, MDA: Malondialdehyde, NO: Nitric oxide, AChE: Acetylcholinesterase, IL-1 β : Interleukin-1 β

Bromelain reduced scopolamine neurotoxicity

DISCUSSION

Oxidative stress, neuro-inflammation and cholinergic dysfunction have been implicated in the pathomechanism of Alzheimer's disease (AD). AD is not just a cognitive disorder, but also associated with pathophysiological and psychological disturbances including motor imbalance, depression and anxiety which are often difficult to manage (Brechtin *et al.*, 2013). Drugs for managing dementia do not relieve the non-cognitive co-morbidities while the antipsychotic drugs used to treat non-cognitive symptoms of dementia put patients at the risk of stroke and sudden death (Kales *et al.*, 2012). Non-pharmacologic interventions like alternative therapies from plant sources may be beneficial as there is increasing interest in the use of natural products which have the advantage of fewer side effects. As such, the present study investigated the neuroprotective effects of bromelain on cognitive, motor and mood (anxiety and depression) imbalance that may be associated with scopolamine induced neurotoxicity and its mechanism(s) of action.

In this study, intervention with bromelain ameliorated scopolamine-induced neurotoxicity by improving cognitive function, exploratory activities and inducing anxiolytic and anti-depressive effects. Furthermore, the effects of bromelain favorably compare with donepezil intervention. This corroborates a recent study wherein bromelain showed similar behavioral profile as ours, though in an aluminum chloride model of AD. However, while the mechanisms underlying their observation were modulations of TXNIP/P-IRS-1/PI3K pathway and AChE inhibition (Eraky *et al.*, 2023), we attribute our results to modulation of oxidonitrosative stress, cholinergic transmission and inflammation. We observed anxiety- and depression-like symptoms in rats exposed to scopolamine. Anxiety and depression are common comorbid emotional deficits associated with dementia which adversely affect the patient's quality of life (Sinoff & Werner, 2003). Anxiety-like behavior is an early sign of AD and can predict future cognitive deterioration in elderly individuals (Donovan *et al.*, 2018). Depression is also a predictor of preclinical cognitive deterioration in AD and is positively correlated with the risk of developing dementia (Byers & Yaffe, 2011). The use of open field, elevated plus maze and forced swimming tests in the analysis of anxiety-like and depressive behavior in rodents are well documented for screening potential anti-depressant and anxiolytic agents (Kremer *et al.*, 2020). Our study design is a little deviation from most studies which often use scopolamine to model only cognitive deficit (Imran *et al.*, 2020; Ayinla *et al.*, 2019; Oladun *et al.*, 2023; Ayinla *et al.*, 2020), with the exception of very few studies that showed emotional disturbance following scopolamine exposure (Jafarian *et al.*, 2019; Cheon *et al.*, 2021). Anxiety and depression are common in dementia including Alzheimer's disease. A single or repeated administration of scopolamine to rodents at a dose that can induce cognitive impairment can in turn, induce anxiety- and depressive-like behaviors (Aydin *et al.*, 2016; Rahmati *et al.*, 2017) primarily by acting as a muscarinic acetylcholine receptor antagonist, disrupting the cholinergic system in the brain, which is crucial for cognitive function and mood regulation. Scopolamine blocks muscarinic acetylcholine receptors, disrupting the

normal signaling pathways involve in learning, memory and mood regulation (Popovic *et al.*, 2015). However, pretreatment with 50mg/kg dose of bromelain effectively prevented the anxiety-like and depressive-like behavior induced by scopolamine exposure suggesting that bromelain could be used as a prophylactic agent to manage mood disorder. This is in tandem with the report of Bakare & Owoyele (2021) wherein, a similar dose of bromelain reportedly attenuated anxiety and depressive-like comorbidity in a rat model of neuropathic pain. An in-vitro study reported that bromelain promoted the degradation of amyloid β 1–42 monomers and soluble aggregates in the cerebrospinal fluid from patients with AD (Sancesario *et al.*, 2018). Being an in-vitro study, there were no behavioral evidence to further validate this report. We also showed that bromelain improved memory index in the novel object recognition test (NORT) which further affirms earlier studies that reported that juice and ethanolic extract of pineapple significantly restored object recognition ability in mice treated with scopolamine. Similarly, peel extract of pineapple significantly attenuated high fat diet-induced reduction in correct alternation in Y-maze test and discrimination index in NORT (Ajayi *et al.*, 2021). These findings suggest that pineapple has a protective role against cognitive disorders (Momtazi-borojeni *et al.*, 2017). Similarly, bromelain was observed to be beneficial in healthy animals compared with control group. The translational implication of this finding is that bromelain could be consumed by healthy individuals or in non-pathological states to prevent the impacts of disorders associated with neuro-inflammation and oxidative stress. This observation corroborates the findings of (Bakare & Owoyele, (2021) and Bayo-Olugbami *et al.* (2024).

In addition to anxiety, the open field and horizontal bar tests showed that exploratory activity, rearing and movement on bar were decreased following scopolamine exposure. The state of anxiety is a typical reactionary response to dementia (Sinoff & Werner, 2003). The degree of anxiety is conventionally linked with the state of alertness during exploration in a novel environment (Ferdman *et al.*, 2007). Therefore, the suppressed rearing and exploration is thought to have resulted from a feeling of apprehension, resulting from anxiety (Adu *et al.*, 2022).

Acetylcholine is the major neurotransmitter that acts on widely distributed cholinergic receptors in the brain, and appropriate cholinergic neurotransmission is fundamental to learning and the formation of memory (Donovan *et al.*, 2014). AChE hydrolyzes acetylcholine, thus diminishing cholinergic neurotransmission. Hence, the assessment of the in vitro and ex vivo activity of AChE proves valuable to assess cholinergic activity in the brain and can be correlated with cognitive function. The non-selective anti-muscarinic drug, scopolamine, passes through the blood-brain barrier (BBB) and mimics the memory impairment symptoms of AD and aging in rats by impairing cholinergic neuronal transmission (Lee *et al.*, 2011). In our behavioral studies, scopolamine-induced memory deficit as depicted by reduced recognition index is a pointer to the fact that rats failed to show any preference for novelty in the NORT. On the contrary, rats treated with bromelain or donepezil, explored the novel object significantly. In furtherance, biochemical evaluation of the brain (PFC) also revealed that scopolamine-induced elevated AChE activity was reduced

by intervention with bromelain or donepezil, suggesting that inhibition of AChE might be one of the possible mechanisms behind this memory improvement. This agrees with the submission of Eraky *et al.* (2023) but opposes the findings of Chaudhary *et al.* (2018) which reported an increase in AChE level by bromelain following dichlorvos toxicity. Consistent with our results, bromelain was previously shown to decrease hippocampal AChE levels in a mouse model of AD (Kumar *et al.*, 2022). Donepezil is a selective, reversible, and centrally acting AChE inhibitor approved for mild to severe AD. Choline esterase inhibitors are the first class of drugs approved in the US and Europe for symptomatic relief of AD (Szeto & Lewis, 2016) and act by increasing brain ACh levels by preventing its degradation. Improving cholinergic neurotransmission remains a common therapeutic strategy for providing symptomatic relief in mild and moderate stages of AD (Marucci *et al.*, 2021).

An imbalance in the antioxidant-oxidant defense mechanism leads to neurological disturbance and neuronal death (Yoshikawa and Naito, 2002). This imbalance was investigated in the current study by estimating the levels of SOD, MDA & NO, which are indices of oxidative stress. Exposure to scopolamine builds oxidative stress in the brain as evident from reduced SOD, increased NO and lipid peroxidation-marked by elevated MDA. This confirms the findings of Ayinla *et al.* (2019) and Ayinla *et al.* (2020). The brain is known to have high concentration of iron which may be involved in the generation of hydroxyl radicals by iron catalysis. It is rich in oxidizable fatty acids and comparatively low antioxidant resistance, which makes the organ more susceptible to oxidative stress (Chaudhary *et al.*, 2018). Overproduction of ROS deteriorates cellular components of neurons, leading to oxidative stress, inflammation and consequently, increased risk of anxiety, depression and altered learning process. Moreover, the pathogenesis of a range of neurological diseases has a strong association with inflammation and oxidative stress (Salim, 2014). In the present study, treatment with bromelain decreased the levels of MDA and NO while it increased SOD following scopolamine exposure. Habashi *et al.* (2012) reported that bromelain significantly reduced the production of nitric oxide in the rat primary microglia. TBARS and PCC level which are markers of oxidative stress were also decreased by concomitant exposure of dichlorvos and bromelain in another study (Chaudhary *et al.*, 2018). Bromelain is able to diminish oxidative stress by minimizing ROS production and halting protein oxidation. It therefore shows that bromelain exerts its protective action by altering the molecular cascades involving SOD, MDA and NO. This action against oxidative stress can be ascribed to the presence of cysteine, an amino acid with known antioxidant properties. Cysteine is also an important precursor in the production of antioxidant-glutathione, which protects cells from toxins. Thus, the cysteine in bromelain could be responsible for combating the oxidative stress (Piste, 2013).

Many studies have shown that sustained inflammation plays an important role in the progression of neurodegenerative disorders. Upregulation of central and peripheral pro-inflammatory cytokines have been implicated as neurobiological molecules that mediate the emotional comorbidity of dementia (Koprich *et al.*, 2008). IL-1 β

receptors are located all over the brain, especially in the PFC, a brain region involved in cognitive, anxiogenic and depressive phenotypes (Lotrich, 2015). Our study shows that scopolamine promotes the elevation of central inflammatory mediator, IL-1 β while treatment with bromelain inhibited it. This further confirms the anti-inflammatory property of bromelain as earlier reported (Adu *et al.*, 2020; Bakare & Owoyele, 2021; Ajayi *et al.*, 2021). Furthermore, our finding suggests that IL-1 β might be responsible for the anxiety and depression associated with scopolamine induced neurotoxicity. Therefore, we suggest that the anti-anxio-depressive-like effects of bromelain may be due in part to its inhibitory effect on central IL-1 β .

Taken together, to the best of our knowledge, this study is the first to demonstrate cognitive and non-cognitive effects of scopolamine in rat and the neuroprotective impacts of bromelain. Hence, we suggest bromelain as a potential natural source of functional food component that may be useful in preventing cognitive and neuropsychiatric disorders. However, we identify with the limitation of our study as we could not compare the observed anxiolytic and anti-depressive effect of bromelain with a reference drug used in managing anxiety and depression.

In conclusion, Bromelain compared favorably with donepezil in improving memory decline and other non-cognitive alterations associated with scopolamine exposure. This could have resulted from modulation of oxidative stress, cholinergic transmission and inflammation. Since bromelain is reportedly safe, its regular consumption is encouraged as a protective protocol against cognitive decline associated with neurodegenerative disorders and mood deficiencies.

REFERENCES

- Adu T, Adu O & Mabandla M (2022). Effects of Bromelain on Noradrenaline Release During Anxiety-Like Behaviour Following Intra-Medial Forebrain Bundle 6-OHDA Injection in Rat Model of Parkinsonism. *Surg Res*, 4(2):1-7
- Agarwal S, Chaudhary B, Bist R (2016). Bacoside A and bromelain relieve dichlorvos induced changes in oxidative responses in mice serum, *Chem. Biol. Interact.* 254: 173–178.
- Ajayi AM, John KA, Emmanuel IB, Chidebe EO, Adedapo DAA (2021). High-fat diet-induced memory impairment and anxiety-like behavior in rats attenuated by peel extract of Ananas comosus fruit via atheroprotective, antioxidant and anti-inflammatory actions. *Metabolism Open* 9:100077
- Aydin E, Hritcu L, Dogan G, Hayta S, Bagci E (2016). The effects of inhaled Pimpinella Peregrine Essential Oil on Scopolamine-induced Memory Impairment, Anxiety and depression in Laboratory Rats. *Mol Neurobiol.* 53 (9):6557-6567
- Ayinla M T, Asuku A O, Bayo-Olugbami A, Ayeni O A, Abiola A A and Owoyele B V (2020). Melatonin and Vitamin C modulate cholinergic neurotransmission and oxidative stress in scopolamine-induced rat model of memory impairment *J. Afr. Ass. Physiol. Sci.* 8 (1): 50-58
- Ayinla, M. T., Uthman, Y., Bayo-Olugbami, A., Oyewole, A. L. and Owoyele, B. V (2019). Effects of Ethylacetate Leaf Extracts of Ocimum gratissimum and Momordica charantia on Memory in Scopolamine-induced Dementia Rats. *Nigerian Journal of Biochemistry and Molecular Biology*; 34(1&2): 28-37

- Bakare A.O & Owoyele B.V (2021). Bromelain reduced pro-inflammatory mediators as a common pathway that mediate antinociceptive and anti-anxiety effects in sciatic nerve ligated Wistar rats. *Scientific Reports* 11:289. doi.org/10.1038/s41598-020-79421-9
- Bakare, A. O. & Owoyele, B. V (2020). Antinociceptive and neuroprotective effects of bromelain in chronic constriction injury-induced neuropathic pain in Wistar rats. *Korean J. Pain* 33, 13–22
- Bayo-Olugbami A, Nafiu AB, Amin A, Ogundele OM, Lee CC & Owoyele BV (2020). Vitamin D attenuated 6-OHDA-induced behavioural deficits, dopamine dysmetabolism, oxidative stress, and neuro-inflammation in mice, *Nutritional Neuroscience*, DOI:10.1080/1028415X.2020.1815331
- Bayo-Olugbami, A.A., Muritala, A.K., Abdur-Rahman, H., Bakare, A.O., Arogundade, T.T, Atere, T.G. and Owoyele, B.V. (2024). Bromelain administration ameliorates neurobehavioural deficits mediated by cadmium neurotoxicity via oxido-nitrosative stress, cholinergic and neuro-inflammatory modulations in male Wistar rats. *Nig. J. Neurosci.* 15(2). <https://doi.org/10.47081/njn2024.15.2/001>
- Bevins RA and Besheer J (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study recognition memory. *Nat Protoc.* 1: 1306–11.
- Bhui K, Prasad S, George J, Shukla Y (2009). Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway. *Cancer Lett*; 282, 167–176.
- Bist R, Chaudhary B, Bhatt D K (2021). Defensive proclivity of bacoside A and bromelain against oxidative stress and AChE gene expression induced by dichlorvos in the brain of *Mus musculus*, *Sci. Rep.* 11 (1): 3668.
- Blokland, A. (2005). Scopolamine-induced deficits in cognitive performance: A review of animal studies. *Scopolamine Review.* 1: 1-76.
- Bradford M.M (1976). A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 72:248–254
- Brechin D *et al.* (2013). Alternatives to antipsychotic medication: psychological approaches in managing psychological and behavioural distress in people with dementia. Leicester: *British Psychological Society*,
- Byers AL & Yaffe K (2011). Depression and risk of developing dementia, *Nat. Rev. Neurol.* 7 (6):323–331.
- Chaisakdanugull C, Theerakulkait C, Wrolstad R E (2007) Pineapple juice and its fractions in enzymatic browning inhibition of banana. *J. Agric. Food Chem.* 55 (10): 4252–4257.
- Chakraborty AJ, Mitra S, Tallei TE, Tareq AM, Nainu F, Cicia D, Dhama K, Emran TB, Simal-Gandara J, Capasso R (2021). Bromelain a Potential Bioactive Compound: A Comprehensive Overview from a Pharmacological Perspective. *Life* 11, 317.
- Chaudhary B & Agarwal S, R (2018). Invulnerability of bromelain against oxidative degeneration and cholinergic deficits imposed by dichlorvos in mice brains. doi.org/10.1007/s11515-018-1479-1
- Cheon SY, Koo BN, Kim SY, Kam EH, Nam J & Kim EJ (2021). Scopolamine promotes neuroinflammation and delirium-like neuropsychiatric disorder in mice. *Sci Rep.* 11:8376
- Donovan NJ, Amariglio RE, Zoller AS, Rudel RK, Gomez-Isla T, Blacker D, *et al* (2014). Subjective cognitive concerns and neuropsychiatric predictors of progression to the early clinical stages of Alzheimer disease. *Am J Geriatr Psychiatry.* 22:1642–51. doi: 10.1016/j.jagp.2014.02.007
- Donovan NJ, Locascio JJ, Marshall GA, Gatchel J, Hanseuw BJ, Rentz DM, Johnson KA, Sperling RA (2018). Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults, *Am. J. Psychiatry* 175 (6):530–537.
- Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharm.* 7(2):88-90.
- Eraky S M, Ramadan N M, Abo El-Magd N F (2023). Ameliorative effects of bromelain on aluminum-induced Alzheimer's disease in rats through modulation of TXNIP pathway. *International Journal of Biological Macromolecules* 227 (2023) 1119–1131. <https://doi.org/10.1016/j.ijbiomac.2022.11.291>
- Ferdman N, Murmu R, Bock J, *et al* (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. *Behavioural brain research*, 180: 174-182.
- Fernando W.M.A.D.B., Martins I.J., Goozee K.G., Brennan C.S., Jayasena V & Martins R.N. (2015) The role of dietary coconut for the prevention and treatment of Alzheimer's disease: Potential mechanisms of action. *Br J Nutr.* 114:1–14.
- Filarowska J, Silberring J, Kotlinska J H (2016). Cholinesterase inhibitors, donepezil and rivastigmine, attenuate spatial memory and cognitive flexibility impairment induced by acute ethanol in the Barnes maze task in rats, *Naunyn Schmiedeberg's Arch. Pharmacol.* 389 (10): 1059–1071.
- Habashi S A, Moghimi A, Sabouni F, Majd S A (2012). Inhibition of NO production in LPS-stimulated primary rat microglial cells by Bromelain. *J of Cell and Mol Res*, 3(2): 57–65
- Haider, S., Tabassum, S., Perveen, T. (2016). Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: a comparative study. *Brain Research Bulletin* 127: 234–247.
- Imran I, Javaid S, Waheed A, Rasool MF, Majeed A, Samad N, Saeed H, Alqahtani F, Ahmed MM and Alaql FA (2021) Grewia asiatica Berry Juice Diminishes Anxiety, Depression, and Scopolamine-Induced Learning and Memory Impairment in Behavioral Experimental Animal Models. *Front. Nutr.* 7:587367. doi: 10.3389/fnut.2020.587367
- Jafarian S, Ling KH, Hassan Z, Perimal-Lewis L, Sulaiman MR, Perimal EK (2019). Effects of Zerumbone on scopolamine-induced memory impairment and anxiety-like behaviours in rats. *Alzheimers Dement.* 5:637-643
- Kales HC *et al.* (2012). Risk of mortality among individual antipsychotics in patients with dementia. *Am J Psychiatry*; 169: 71-9.
- Kim EJ, Jung IH, Jeong JJ, Kim NJ, Kim DH (2013). Ginsenosides Rg5 and Rh3 protect scopolamine-induced memory deficits in mice. *Journal of Ethnopharmacology* 146: 294-299.
- Koprach JB, Reske-Nielsen C, Mithal P, Isacson O (2008). Neuroinflammation mediated by IL-1 β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease, *J. Neuroinflamm.* 5:8–14.
- Kremer M, Becker LJ, Barrot M & Yalcin I (2020). How to study anxiety and depression in rodent models of chronic pain? *Eur. J. Neurosci.* 2020, 1–35.

- Kumar R, Kumar R, Sharma N, Khurana N, Singh SK, Satija S, Mehta M, Vyas M (2022). Pharmacological evaluation of bromelain in mouse model of Alzheimer's disease, *NeuroToxicology* 90:19–34.
- Lee B, Shim I, Lee H, Hahm DH (2011). Rehmannia glutinosa ameliorates scopolamine-induced learning and memory impairment in rats. *J Microbiol Biotechnol.* 21:874–83
- Lister RG (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92:180e5.
- Livingston G et al., (2005). Systematic review of psychological approaches to the management of neuropsychiatric symptoms of dementia. *Am J Psychiatry*; 162: 1996–2021.
- Lotrich FE (2015). Inflammatory cytokine-associated depression. *Brain Res*, 1617:113e25.
- Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F (2021). Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease, *Neuropharmacology* 190:108352.
- Momtazi-borojeni AA, Sadeghi-Aliabadi H, Rabbani M, Ghannadi A & Abdollahi E (2017). Cognitive enhancing of pineapple extract and juice in scopolamine-induced amnesia in mice. *Research in Pharmaceutical Sciences*, 12(3): 257–264.
- Mostafa, N.M.; Mostafa, A.M.; Ashour, M.L.; Elhady, S.S (2021). Neuroprotective Effects of Black Pepper Cold-Pressed Oil on Scopolamine-Induced Oxidative Stress and Memory Impairment in Rats. *Antioxidants*, 10:1993. <https://doi.org/10.3390/antiox10121993>
- Ohkawa, H., Ohishi, N., Yagi, K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*. 95(2):351–8.
- Oladun B T, Oyerinde A, Oyadeyi A S, Badmus H A, Onasanwo A S (2023). Effect of Cocos nucifera L. Water on Scopolamine-Induced Memory Impairment in the Wistar Rat. *Nig J Neurosci* 14(1): 17–25
- Piste P. (2013) Cysteine–Master Antioxidant. *Int. J. Pharmaceut. Chem. Bio. Sci.* 3, 143–149.
- Popovic M, Gimenez de Bejar V, Popovic N, Caballero-Bleda M (2015). Time course of scopolamine effect on memory consolidation and forgetting in rats. *Neurobiol Learn Mem.* 118: 49–54
- Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *European Journal of Pharmacology*, 47(4), 379–391. doi.org/10.1016/0014-2999(78)90118-8
- Rahmati B, Kiasalari Z, Roghani M, Khalili M, Ansari F (2017). Antidepressant and anxiolytic activity of Lavandula Officinalis aerial parts hydroalcoholic extract in scopolamine-treated rats. *Pharm Biol*, 55(1): 958–965
- Salim S (2014). Oxidative stress and psychological disorders. *Curr Neuropsychopharmacol.* 12:140–7
- Sancesario GM, Nuccetelli M, Cerri A, Zegeer J, Severini C, Ciotti MT, Pieri M, Martorana A, Caltagirone C, Nistico R, Bernardini S (2018). Bromelain degrades A β 1–42 monomers and soluble aggregates: an in vitro study in cerebrospinal fluid of Alzheimer's disease patients, *Curr. Alzheimer Res.* 15 (7):628–636.
- Sinoff G & Werner P (2003). Anxiety disorder and accompanying subjective memory loss in the elderly as a predictor of future cognitive decline, *Int. J. Geriatr. Psychiatry* 18 (10):951–959.
- Szeto JY & Lewis SJ (2016). Current treatment options for Alzheimer's disease and Parkinson's disease dementia, *Curr. Neuropsychopharmacol.* 14 (4):326–338.
- Tochi B.N, Wang Z, Xu S.Y, Zhang W (2008). Therapeutic application of pineapple protease (Bromelain): A review. *Pakistan J. Nutr.* 7, 513–520.
- Tracey, W.R., J. Tse, and G. Carter (1995) Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. *J Pharmacol Exp Ther.* 272(3): 1011–5.
- Yankelevitch-Yahav R, Franko M, Huly A, Doron R. (2015). The forced swim test as a model of depressive-like behavior. *J Vis Exp.* e52587.
- Yoshikawa T & Naito Y (2002). What Is Oxidative Stress? *J of the Japan Med Association*, 45(7): 271–2.

Full length Research Article

Cognitive and Neuroprotective Effects of *Vernonia amygdalina* in Scopolamine-induced Memory impaired Rats

*Odu P.O.¹, Odu V.K.¹, Oyebanjo O.T.², Benneth B.³, and Onasanwo S.A.⁴

¹Department of Physiology, Faculty of Basic Medical Sciences, Calabar, Calabar, Nigeria

²Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria

³Department of Physiology, Babcock University, Nigeria

⁴Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.

Summary: Cognitive impairment is largely associated with functional and structural loss in the brain of Alzheimer's disease (AD) models, and scopolamine has been successfully used to mimic these deficits in rodents. The cost and side effects of drugs presently used for the treatment of AD-related cognitive impairment have prompted research into alternative products, especially natural ones with high antioxidant capacity since oxidative stress is a major pathophysiology associated with AD. The current study evaluated the cognitive and neuroprotective effects of *Vernonia amygdalina* (VA) on scopolamine-induced cognitive impairment in rats. Thirty-five male rats were randomly divided into seven groups (n = 5). Group 1 served as the control and received distilled water. Groups 2 and 3 received *Vernonia amygdalina*, VA (50 and 100 mg/kg, respectively) per orally (p.o.). Group 4 received 1 mg/kg scopolamine SC (i.p.). Groups 5, 6, and 7 received pretreatment with either VA 50 mg/kg, VA 100 mg/kg, or Donepezil, DP (1 mg/kg), and then in combination with SC (1 mg/kg). The animals were subjected to memory tasks using the Morris water maze (MWM) and novel object recognition tasks (NORT). They were sacrificed on day 14, after which their brains were isolated for biochemical and histological studies. The study showed that during MWM and NORT, spatial and non-spatial recognition memories, respectively impaired in the SC group compared to the control group, were reversed in the VA pretreatment groups. Scopolamine injection caused significant decreases in superoxide dismutase and catalase levels and an increase in malonaldehyde (MDA) levels in group 4 compared with the control group. Pretreatments with either VA or DP, however, caused a significant increase in the SOD and catalase levels and a decrease in the MDA level compared with the SC group. Histological studies revealed that VA was more potent in protecting the brain against SC-induced neurodegeneration and morphological alterations in the hippocampus and prefrontal cortex. The findings of this study suggest that VA attenuates scopolamine-induced cognitive deficits via inhibition of oxidative stress and neuronal degeneration and enhancement of cognition in the brains of rats.

Keywords: Cognitive deficits, Alzheimer disease, *Vernonia amygdalina*, Scopolamine, Memory, Oxidative stress.

*Authors for correspondence: : odupeterori@unical.edu.ng, odupeterori@gmail.com, Tel: +234-8036865949

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INTRODUCTION

Cognitive functions are multiple mental attributes that enable humans and associated organisms to effectively interact with their environment, carry out daily tasks, and adapt to different survival strategies. Amongst these are learning and memory which predominantly have been reported to show a decline in efficiency as a person ages, and in disease conditions like Alzheimer's disease (AD). AD is an age-related, progressive neurodegenerative disorder of the central nervous system, the most common form of dementia characterized by cognitive dysfunction, altered personality, loss of executive function, and ultimately death (Xingxuan *et al.*, 2008; Merlo *et al.*, 2010). Investigations of the brains of AD patients show low levels of neuronal antioxidant defense enzymes, elevated levels of

oxidative stress biomarkers, and increased levels of reactive oxygen species (ROS) and nitrogen species (Walton *et al.*, 2012). Preclinical and clinical studies have revealed extensive neuronal loss and synaptic changes in the basal forebrain, particularly the areas involved in learning and memory (Giacobini, 2003). Scopolamine, an M₁ muscarinic receptor subtype has been experimentally used to induce AD-related disorders in animals. Tota *et al.* (2012) reported that scopolamine impairs memory through mechanisms that increase acetylcholinesterase and induce oxidative stress in the brain. Drugs like donepezil and tacrine currently used to manage AD, are associated with short half-lives, poor bioavailability, and serious side effects, including hepatotoxicity (Rountree *et al.*, 2009). Thus, there is growing research interest in the use of alternative remedies, such as plants with medicinal, antioxidant, and therapeutic

potential, for the treatment of AD-related cognitive deficits. Dietary polyphenols have gained significant attention due to their rich antioxidant and therapeutic potential in reducing somatic cell and neuronal damage (Engelhart *et al.*, 2002). *Vernonia amygdalina* (VA) leaves, commonly known as bitter leaf, have been widely used for both therapeutic and nutritional purposes (Ojiako and Nwanjo, 2006). VA possesses strong polyphenolic, antioxidant, anxiolytic, antinociceptive, and anti-inflammatory (Iwalokun *et al.*, 2006; Onasanwo *et al.*, 2017) properties. It is an abundant source of linoleic and linolenic acids, and polyunsaturated fatty acids found to be protective against cardiovascular diseases (Tapsell, 2006). This study was therefore designed to investigate the cognitive and neuroprotective effects of VA leaf extracts in scopolamine-induced cognitive impairment in Wistar rats.

MATERIALS AND METHODS

Experimental animals: Male Wistar rats (80-120 g) obtained from the central animal house of the University of Ibadan, were used for this study. The rats were housed in plastic cages at room temperature and given free access to commercial food pellets and water *ad libitum*. They were acclimatized for two weeks before commencement of experiments. The experimental procedures were carried out in accordance with the guidelines for animal research as detailed in the guidelines for the Care and Use of Laboratory Animals by the National Research Council of the National Academy of Sciences, 2011.

Plant and chemicals: *Vernonia amygdalina*, Scopolamine hydrobromide (Sigma-Aldrich.co., St. Louis, U.S.A), and Donepezil hydrochloride (torrent pharma, UK Ltd.) were used in the study.

Plant Extraction using Soxhlet technique: Fresh mature leaves of VA were obtained and verified at the University of Ibadan. The leaves were air-dried and blended into powder form using an electric blender. Its weight (2600 g) was determined using an electric weighing balance and then soaked in 80% (v/v) methanol for 72 hours at a temperature of 40°C. The extract was filtered using a cheese material, and thereafter with Whatman No 1 filter paper. The filtrate was concentrated to dryness using a rotary evaporator.

Experimental design and treatments: The rats were divided into 7 groups (n = 5). Group 1 received distilled water (per orally, *p.o*) alone throughout the experiment, from day 1-14 and served as the Control (CT). Group 2 and 3 received *Vernonia amygdalina*, VA (50 and 100 mg/kg respectively) alone (*p.o*) for 14 days. Group 4 received distilled water (*p.o*) from day 1-7 as well as 1 mg/kg scopolamine SC (*i.p*) from day 8-14. Groups 5, 6 and 7 were pretreated with *Vernonia amygdalina* (SC+VA 50 mg/kg), (SC+VA 100 mg/kg), and Donepezil (SC+DP 1 mg/kg) respectively, *p.o* for 7 days, and then in combination with scopolamine (1 mg/kg) (*i.p*) one hour later from day 8-14.

Behavioral assessments

Novel object recognition task (NORT): On the experimental day, animals underwent two sessions, each

consisting of a single trial. In the initial session, referred to as the acquisition trial (trial 1), animals were placed in an arena with two identical objects for 5 minutes. Any rat that did not engage with the objects for at least 20 seconds within the 5-minute period were excluded from the experiments. Exploration was defined as the animal bringing its nose within 1 cm of the object while looking at, sniffing, or touching it. In the second session, known as the retrieval session (trial 2), which occurred 24 hours after trial 1, one of the objects used in the first trial was replaced by a new, unfamiliar object (the novel object). Animals were once again placed in the arena for 5 minutes, during which their total exploration time for each were recorded and the time spent actively exploring the familiar (F) or novel (N) object in trial 2. Olfactory cues were eliminated in between trials by cleaning the test box with 70% of ethanol. Percentage exploratory preference which served as index non-spatial recognition memory was evaluated using the formula: $[(N)/(N+F)] \times 100$ (Bevins and Besheer, 2006).

Morris water maze (MWM): The MWM apparatus used in this study was made of a circular GP water tank (150 cm diameter and 74 cm height) filled with water ($25 \pm 2^\circ\text{C}$) to a depth of 30 cm. The pool was divided into four hypothetical quadrants, designated as: East, West, North, and South. Throughout the trial, a white round platform of diameter 10 cm was placed 2 cm below the surface of the water in a constant position; in the middle of the southwest quadrant. The water was made opaque by adding a food coloring (pink milk) in order to prevent the animal from noting the depth of the water. Animals were placed 1 inch above the water, positioned to face the wall of the pool. In each trial rats were given a maximum of 60 s cut-off time to locate the hidden platform. This was recorded using a stopwatch, and then averaged for each training session. In the event that the animal was unable to locate the hidden platform within 60 s, it was gently guided to it, and allowed to stay there for 30 s. Each rat was subjected to a daily session of 2 trials for 3 consecutive days, while the animals were allowed to rest on the 4th day. On day the 5th day, the platform height was further lowered below the water level, and the rats underwent a spatial *probing* trial in which they were given 60 s to search for the platform. Escape latency time (ELT) which was the time taken to locate the hidden platform in the water maze was used as an index of spatial learning and memory (Morris, 1984; Ishola *et al.*, 2013).

Preparation of Brain Homogenate: On the 14th day of the protocol schedule, animals underwent cervical dislocation. Brains were removed and rinsed with ice-cold isotonic saline solution. Brain tissue samples were then homogenized with 10 times (w/v) ice-cold 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min to obtain the supernatant. The supernatant was collected and separated into different portions that were kept refrigerated for the different biochemical assays.

Biochemical estimations

Estimation of Protein concentration: The protein concentration of brain homogenate was determined by the Biuret method described by Gornal *et al.*, (1949).

Estimation of Malondialdehyde (MDA): MDA, an end product of lipid peroxidation was measured by the method described by Varshney and Kale (1990), and expressed in units/mg protein.

Estimation of Superoxide Dismutase (SOD) Activity: SOD activity was estimated by the method described by Misra and Frodovich (1972) based on the inhibition of Epinephrine-Adrenochrome transition by the enzyme. The enzyme activity was expressed in terms of units/mg protein.

Estimation of Nitrite Level: Nitrite level which served as an indicator of nitric oxide (NO) production was estimated using the Greiss reaction as described by Green *et al.* (1982).

Histological Studies: Some of the animals were perfused with 0.01 M phosphate buffer solution (PBS, pH 7.4) followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed and post-fixed in 4% formaldehyde for histological study. The tissues were observed and cut into small pieces not more than 4mm thick into pre-labeled cassettes. The brains were removed and placed in a new formaldehyde solution for 24 h to be fixed before being dehydrated using alcohol (70% for 24 h 80% for 1 h and 95% for 1 h absolute 1 and absolute 11, for 1 hr) then cleared in xylene and embedded in paraffin. Sections were cut with a microtome (Leica TP1020, Germany) at 5 μ m thicknesses, mounted on glass slides, and stained with the routine haematoxylin and eosin (H&E) technique stain, and Cresyl fast violet (Nissl). Staining was done as described by Avwioro (2010). Examination of slides, photography, and morphometric studies were done at the Histopathology Department, University Teaching Hospital, Ibadan. Good sections from each group were signed and documented as photomicrographs using Olympus digital camera.

Statistical Analysis: All the data were expressed as mean $\pm$ standard error of mean, (n=5). Data were analyzed using one- or two-way ANOVA followed by Turkey post hoc test (where applicable) to determine the level of significance. Analysis was done using GraphPad prism software, version 7 (Diego, CA, USA).

RESULTS

Comparison of percentage exploratory preference (PEP) during NORT in rats: One-way ANOVA showed significant differences in the PEP across the groups. Post hoc analysis showed that PEP significantly decreased in the SC group ($p<0.0001$) compared with the control group. However, VA inhibited the effects of scopolamine-induction in the pretreatment groups during NORT test (Fig. 1).

Comparison of escape latency time (ELT) during acquisition trials in MWM: One-way ANOVA showed that there were no significant differences in the ELT across the groups during MWM acquisition trials of day 10, 11, and 12. Post hoc analysis showed progressive decreases in ELT in all the groups, except in the group that received scopolamine injection alone (Fig. 2)

Comparison of ELT during probe trial in MWM: One-way ANOVA showed significant differences in the ELT to access spatial memory across the groups. Post hoc analysis

revealed that ELT was significantly increased in the SC group which received scopolamine injection alone ($p<0.0001$) and in the SC+VA 50 mg/kg ($p<0.01$) compared with the control group. ELT was however significantly decreased SC+VA 50 mg/kg ($p<0.001$) and SC+DP 1 mg/kg ($p<0.01$) compared with the SC group (Fig. 3).

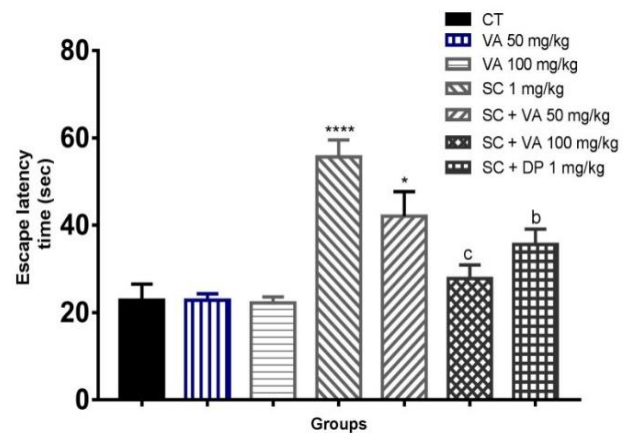


Figure 1. PEP during NORT. Values are expressed as mean $\pm$ SEM, n = 5. **** $p<0.0001$ versus Control (CT); ^b $p<0.0001$ versus scopolamine (SC) treated.

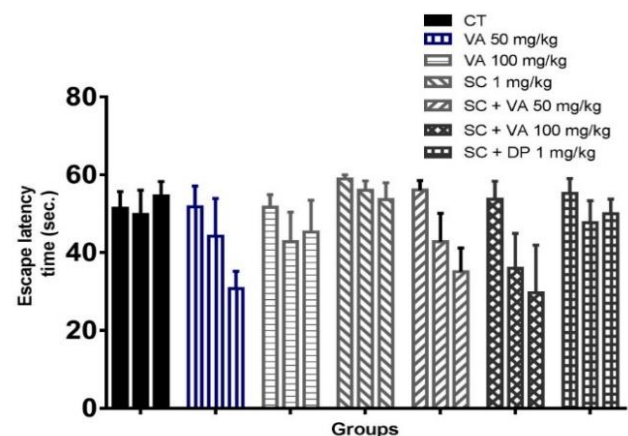


Figure 2. Escape latency during acquisition trial in MWM. Values are expressed as Mean $\pm$ SEM, n = 5.

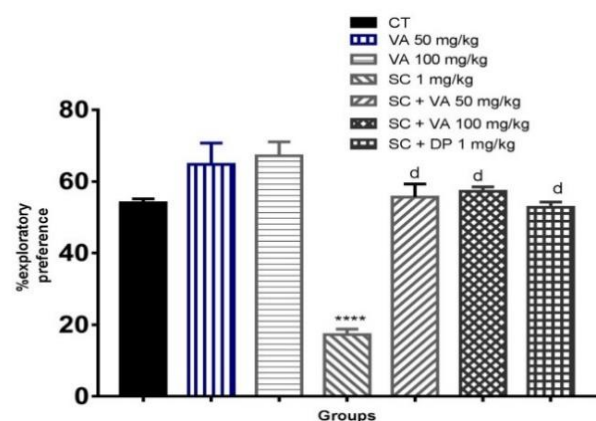


Figure 3 Escape latency during probe trial in MWM. Values are expressed as mean $\pm$ SEM, n = 5. * $p<0.05$ and **** $p<0.0001$ vs CT; ^b $p<0.01$ and ^c $p<0.001$ vs SC treated.

Comparison of protein concentration in experimental rats: As shown in Fig. 4, one-way ANOVA showed that scopolamine injection produced significant changes in protein concentration. Post hoc analysis showed that Protein concentration decreased significantly ($p<0.001$) in the SC group compared with the control. However, the SC+VA (50 and 100 mg/kg) and SC+DP pretreatments groups showed significant decreases ($p<0.0001$ and $p<0.00$) and ($p<0.01$) respectively compared with the SC group (fig. 4).

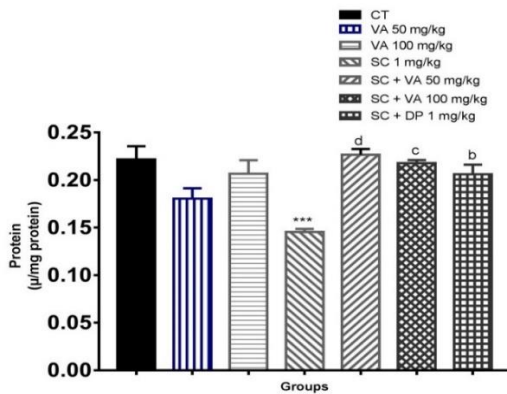


Figure 4.

Effects of VA on protein concentration. Values are expressed as mean $\pm$ SEM, $n = 5$. *** $p<0.001$ vs CT; ^b $p<0.01$, ^c $p<0.001$ and ^d $p<0.0001$ vs SC treated.

Comparison of Malonaldehyde (MDA) levels in experimental rats: Fig. 5 shows the effects of scopolamine injection caused significant changes in the MDA levels in rats' brains as revealed by one-way ANOVA. Post hoc analysis showed that MDA levels significantly increased in the SC ($p<0.0001$), SC+VA 100 mg/kg ($p<0.05$), and SC+DP ($p<0.001$) groups compared with the CT group. While MDA significantly decreased in the SC+VA (50 mg/kg) compared with the SC group, it also decreased significantly in SC+VA 100 mg/kg ($p<0.0001$), and SC+DP ($p<0.001$) groups compared with the SC group (fig. 5).

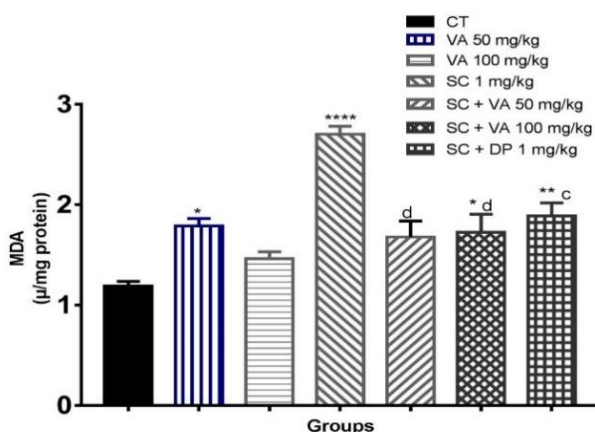


Figure 5.

Effects of VA on MDA level. * $p<0.05$ ** $p<0.01$ and **** $p<0.0001$ vs CT; ^c $p<0.001$ and ^d $p<0.0001$ vs SC treated.

Comparison of Nitrite levels in experimental rats

One-way ANOVA showed significant differences in nitrite levels across the groups. Post hoc analysis showed that nitrite level was significantly increased in SC+VA (50 and 100 mg/kg) groups ($p<0.001$, $p<0.05$, respectively), and SC

group ($p<0.0001$) groups compared with the CT group. Nitrite levels also increased significantly in the SC+VA (50 and 100 mg/kg) or SC+DP groups ($p<0.0001$, $p<0.001$, $p<0.01$, respectively) compared with the control. However, pretreatment of rats with VA inhibited the severity of scopolamine-induced effects by significant decreases ($p<0.0001$) in the Nitrite levels across the combination groups (Fig. 6).

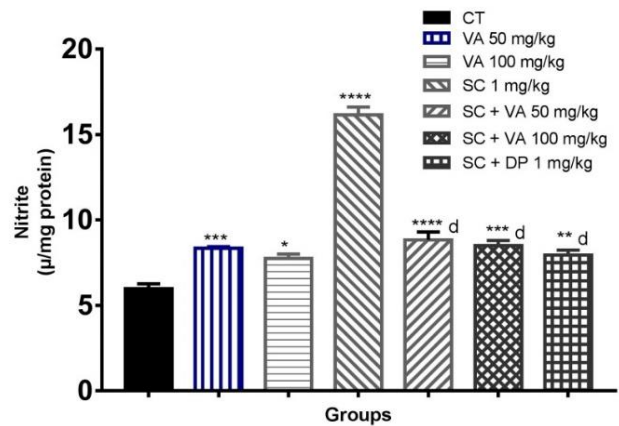


Figure 6

Effects of VA on Nitrite level. Values are expressed as mean $\pm$ SEM, $n = 5$. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ vs CT; ^d $p<0.0001$ vs SC treated.

Comparison of SOD level in experimental rats: One-way ANOVA showed significant differences in the SOD level across the groups. Post hoc analysis showed significant decreases in the SOD levels in the SC group ($p<0.0001$) compared with the CT group. SOD also showed significant decreases in the SC+VA (50 and 100 mg/kg) and SC+DP groups ($p<0.05$ and $p<0.05$; and $p<0.01$), respectively compared to the CT group. SOD was however significantly increased SC+VA (50 and 100 mg/kg) and SC+DP groups ($p<0.0001$); and $p<0.001$), respectively compared to SC group (Fig. 7).

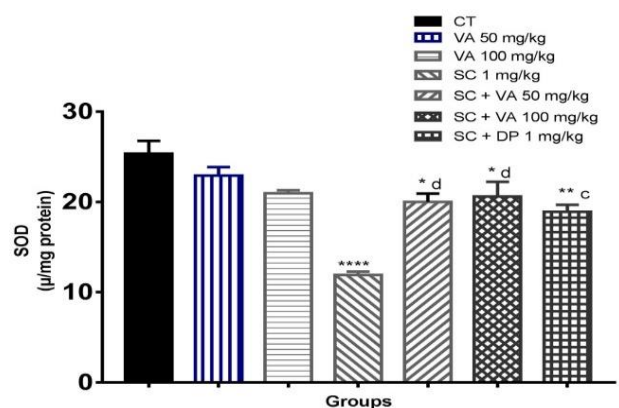


Figure 7

Effects of VA on SOD level. * $p<0.05$, ** $p<0.01$, and **** $p<0.0001$ vs CT; ^c $p<0.001$, and ^d $p<0.0001$ vs SC treated.

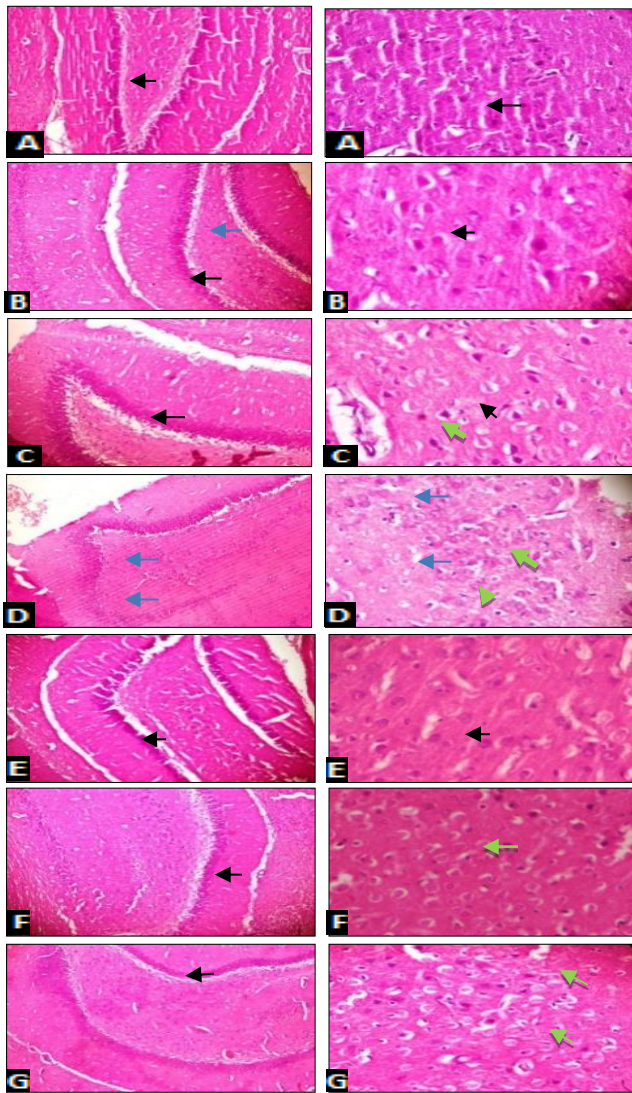


Plate 1a:
Photomicrograph of brain sections of the **hippocampus, hp (left),** and prefrontal cortex, **pc (right) stained with H&E.**

The Plate 1a shows (A) Control rats exhibit a normal histological structure with intact neuronal cells in both the hippocampus (hp) and prefrontal cortex (pc); (B) Rats treated with *Vernonia amygdalina* (VA, 50 mg/kg) display a few degenerated neurons (hp), and normal neuronal cells (pc); (C) Rats treated with VA (100 mg/kg) show normal neuronal cells (hp), and chromatolysis in a few neuronal cells (pc); (D) scopolamine-induced AD rats showing several degenerated neurons (blue arrows) in both **hp and pc**. Degenerated neurons in the pc appear transparent and exhibit chromatolysis; (E) scopolamine-induced AD rats pretreated with VA treatment (50 mg/kg) showing normal neuronal cells (in hp and pc); (F) scopolamine-induced AD rats pretreated with VA treatment (100 mg/kg) display normal neuronal cells (hp), **and** moderate chromatolysis (pc); (G) scopolamine-induced AD rats pretreated with Donepezil (1 mg/kg) exhibit moderately normal neuronal cells (hp), with some degenerated neuronal cells showing degree of hyalinization and chromatolysis (pc).

Plate 1b shows (A) show a normal distribution of Nissl bodies in neurons within the hippocampus (hp) and prefrontal cortex (pc); (B) VA-treated rats (50 mg/kg) show a moderately normal distribution of Nissl bodies (hp), with

a few neuronal cells displaying loss of Nissl bodies (pc); (C) VA-treated rats (100 mg/kg) display moderately normal distribution Nissl bodies (hp), and some neuronal cells with a loss of Nissl bodies in the pc; (D) Scopolamine-induced AD rats exhibit a poor distribution of Nissl bodies (hp), and significant loss of Nissl bodies (red arrows) with a reduced neuronal population (pc). (E) scopolamine-induced AD rats pretreated with VA (50 mg/kg) show moderately restored distribution of Nissl bodies in both hp and pc. (F) Scopolamine-induced AD rats pretreated with VA (100 mg/kg) show a moderate distribution of Nissl bodies, although a few neurons exhibit a loss of Nissl bodies (hp), some neuronal cells in the prefrontal cortex, particularly within the hypocellular layer, display a loss of Nissl bodies (pc); (G) Scopolamine-induced AD rats pretreated with Donepezil (1 mg/kg) exhibit a moderate distribution of Nissl bodies followed by a low neuronal population (hp), there is a reduction in neuronal cells and a significant loss of Nissl bodies (pc).

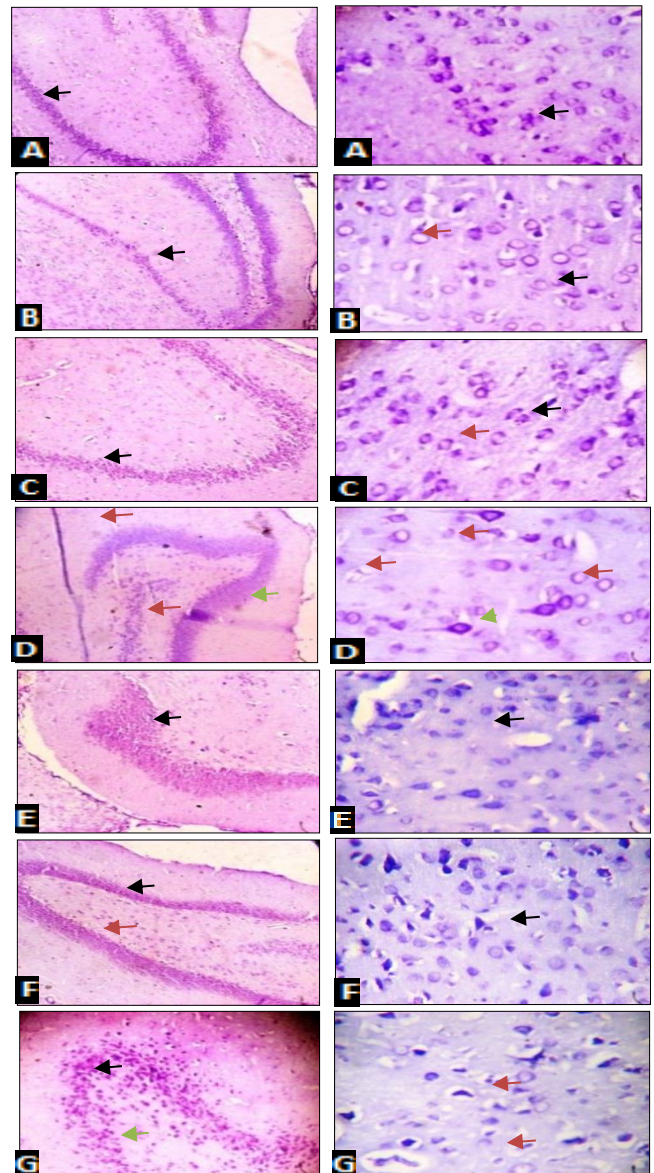


Plate 1b:
Photomicrograph of brain sections of the **hippocampus, hp (left),** and prefrontal cortex, **pc (right) stained with cresyl fast violet.**

DISCUSSION

Our study showed that scopolamine, a non-selective muscarinic receptor antagonist caused significant damages in neuronal architecture in both the prefrontal cortex and the hippocampus with associated cognitive impairment that relates to AD. Scopolamine alters neuronal processes and facilitates neurodegeneration of cortical and hippocampal neurons. Previous studies have reported that scopolamine interferes with cognitive processes involved in memory consolidation and retrievals thus causing short- and long-term memory impairment (Ishola et al., 2013; Souza et al., 2013). Behavioural, neurochemical, and histological changes induced by scopolamine in the current study were attenuated by pretreatment with *Vernonia amygdalina*.

Scopolamine significantly impaired rodents' recognition memory in the novel object recognition task (NORT), a task widely used to assess rodent's long-term, non-spatial recognition memory (Ennaceur, 2010; Pezze et al., 2017). The NORT takes advantage of rodent's unprompted nature to explore its natural surroundings especially novel objects (Ennaceur, 2010). While a higher time spent with the novel object compared to a familiar one within the rodent's environment indicates good residual memory for the familiar object, the opposite indicates memory deficits. Consequently, pre-treatment with *Vernonia amygdalina* (VA) efficiently prevented memory impairment in the NORT when recognition memory was assessed.

Morris water maze (MWM), a widely used neurobehavioral task, was used to assess spatial memory which is a hippocampal-dependent task. Animals were trained to acquire information about a spatial location and reach the hidden platform in the circular pool. Escape latency time (ELT) which is the time taken to reach the hidden platform was significantly increased following scopolamine treatment indicating impairment in memory. Pretreatment with *Vernonia amygdalina* however inhibited and reversed the severe memory deficits recorded in groups that received only scopolamine.

It has also been reported that the central nervous system is very susceptible to oxidative stress (Walton et al., 2012). Biochemical assays in numerous studies relating to AD shows low levels of neuronal antioxidant defense enzymes and elevated levels of oxidative stress biomarkers, which contributes greatly to neuronal damage and cognitive dysfunction (Walton et al., 2012). In a similar context, scopolamine has been reported in several studies to cause memory impairment via oxidative stress pathology in rodents (Khalifa, 2004; Fan et al., 2005).

The current study also shows that scopolamine-induced oxidative stress significantly causes increases in the brain lipid peroxidation with parallel decreases in total protein concentrations (TPC) and superoxide dismutase activity (SOD). However, pretreatment with VA protected the brain from this oxidative stress impact of scopolamine by causing a decrease in Malonaldehyde (MDA) level, an index of lipid peroxidation MDA level, and parallel increases in TPC and SOD. SOD is an anti-oxidant that detoxifies superoxide anions by converting it to hydrogen peroxide and water (Abhinav, 2010). Although previous studies have shown that the central nervous system is very susceptible to oxidative stress (Walton et al., 2012), the VA was able to exert neuroprotective effects. The rich antioxidant

properties of VA have been reported in previous studies (Owolabi et al., 2008). Ogunlade et al. (2012) also reported that SOD and MDA levels significantly improved following *Vernonia amygdalina* treatment in liver oxidative stress caused by alcohol ingestion.

Scopolamine also caused significant elevation in brain Nitric oxide (NO) levels, but this was ameliorated by VA pretreatment. NO is an important oxidative biological signalling molecule with diverse physiological functions including neurotransmission and learning and memory processes (Susswein et al., 2004), however, when generated in excess during oxidative outburst and inflammation, In excess, NO combines with superoxide anion (O_2^-) forming the highly reactive and neurotoxic product peroxynitrite ($ONOO^-$), which leads to further oxidative and nitrosative stress in part via mitochondrial injury (Rao and Balachandran, 2002; Lipton, 2004). Very high level of NO have been implicated in oxidative stress and neuroinflammation in brain parenchyma, neurodegeneration, and neuronal cell death through NO-mediated neurotoxicity (Law et al., 2004; Togo, 2004).

Histological findings in this study support earlier reports that scopolamine extensively destroys neuronal architecture and hippocampal circuits that may play vital roles in learning and memory (Haroutunian et al., 1997; Choi et al., 2012; Chen et al., 2014), as well as depopulates neuronal cells via neurotoxicity (Ramirez-Rodriguez et al., 2011). It has also been reported that scopolamine attenuates memory task-induced increases of regional cerebral blood flow in the prefrontal cortex and the right anterior cingulate region (Grasby et al., 1995). The histological report of the current study showed that scopolamine caused neurodegeneration, chromatolysis, hyalination several losses of nissl bodies in the hippocampus and prefrontal cortex.

Pretreatment with VA however protected the brain from the severity of scopolamine-induced damages. While VA was able to reduce the level of damage induced by scopolamine exposure, there were however other negative side effects across the groups with VA treatment. This may be due to the presence of bioactive compounds such as oxalate previously reported to be insoluble in the methanol form of VA extractions (Nerurkar et al., 2004; Eleyinmi et al., 2006). The presence of oxalate in food has been associated with acidity and toxicity (Eleyinmi et al., 2006). However, studies that involved an aqueous form of *Vernonia amygdalina* extraction recorded no histopathological conditions (Ogunlade et al., 2012; Mebratu et al., 2013). On the other hand, the donepezil treatment group was associated with adverse negative side effects characterized by undifferentiated layers, degeneration, chromatolysis, reduction in neuronal cells, and loss of Nissl bodies, thus making it less effective to protect the brain compared with VA. Moreover, a study by Zaki et al. (2013) reported that donepezil was not strong enough in protecting the brain against scopolamine insults and was associated with some levels of degeneration (Zaki et al., 2013). Donepezil works via a cholinergic pathway to inhibit scopolamine activity and enhance acetylcholine levels which improves cognition. While the scope of this study didn't consider this pathway, VA may be working via the same route to execute its beneficial function.

In conclusion, VA demonstrated neuroprotective effects and improved cognitive deficits induced by scopolamine in

rats thus suggesting a beneficial role in conditions associated with cognitive dysfunctions. Although the cognitive enhancing effect of VA was parallel to that of donepezil, the underlying pathological adverse effects reported in the present and previous studies limit its wide usage. Further research into the mechanism via which VA executes its function may position it as a better alternative to donepezil.

REFERENCES

- Abhinav, K., Jogender, M., Madhusudana, K., Vegi, G., Modi, N., Yogendra, K. and Gupta, R. (2010). Anti-Amnesic Activity of Vitex Negundo in Scopolamine Induced Amnesia in Rats. *Pharmacology & Pharmacy*, 1, 1-8.
- Allam, A.R., Kiran, K., and Hanuman, T. (2008). Bioinformatic analysis of Alzheimer's Diseases using functional protein sequences. *J Proteomics Bioinform*, 1, 036-042.
- Ballard, C.G., Chalmers, K.A., Todd, C., McKeith, I.G., O'Brien, J.T., Wilcock, G., Love, S. and Perry, E.K. (2007). Cholinesterase inhibitors reduce 673 cortical Abeta in dementia with Lewy bodies. *Neurology*, 674 68:1726-1729.
- Bevins, R.A. and Besheer, J. (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nature Protocols*, 1(3):1306-11.
- Bruin N. and Pouzet, B. (2006). Beneficial effects of galantamine on performance in the object recognition task in Swiss mice: Deficits induced by scopolamine. *Pharmacology, Biochemistry and Behavior* 85: 253-260.
- Chen, L., Richardson, J., Caldwell, J. and Ang, L. (1994). Regional brain activity of free radical defense enzymes in autopsy samples from patients with Alzheimer's disease and from non-demented controls, *International Journal of Neuroscience* 75, 83-90.
- Choi, D.Y., Lee, Y.J., Lee, S.Y., Lee, Y.M., Lee, H.H., and Choi, I.S. (2012). Attenuation of scopolamine-induced cognitive dysfunction by obovatol. *Arch Pharm Res.*, 35:1279-86.
- Easton, A., Sankaranarayanan, S., Tanghe, A., Terwel, D., Lin, A. X., Hoque, N., Bourin, C., Gu, H., Ahlijanian, M. and Bristow, L. (2013). Effects of sub- 700 chronic donepezil on brain Abeta and cognition in a mouse model 701 of Alzheimer's disease. *Psychopharmacology*, 230:279-289.
- Eleyinmi, A., Adebowale, Y., Oluwalana, I., Ajisafe, O. and Akintomide, T. (2006). Effect of dietary inclusion of Garcinia kola, Gongronema latifolium and Vernonia amygdalina on the nutritional quality of a complementary diet. *Journal of Biological Sciences.*, 1:43-49.
- Engelhart, M.J., Geerlings, M.I., Ruitenberg, A., van Swieten J.C., Hofman, A., Witteman, J.C., et al. (2002). Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA*. 287:3223-9
- Ennaceur, A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav. Brain. Res.*, 215(2), 244-54.
- Fan Y, Hu J, Li J, Yang Z, Xin X, Wang J, et al. (2005). Effect of acidic oligosaccharide sugar chain on scopolamine-induced memory impairment in rats and its related mechanisms. *Neurosci Lett*; 374(3):222-6.
- Grasby, P.M., Frith, C.D., Paulesu, E., Friston, K.J., Frackowiak, R.S. and Dolan, R.J. (1995). The effect of the muscarinic antagonist scopolamine on regional cerebral blood flow during the performance of a memory task. *Exp Brain Res.*, 104:337-348.
- Giachini, E. (2003). Cholinesterases: new roles in brain function and in Alzheimer's disease. *Neurochemical Research*, 28, 515- 522.
- Gornall, A. G., Barddawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of biuret reaction. *J Bio Chem.*, 177(2): 751-66.
- Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem* 126(1):131-8
- Guo, H. B., Cheng, Y. F., Wu, J. G., Wang, C. M., Wang, H. T., Zhang, C., Qiu, Z. K. and Xu, J. P. (2015). Donepezil improves learning and memory deficits in app/ps1 mice by inhibition of microglial activation. *Neuroscience* 30, 20-30.
- Haroutunian, V., Greig, N., Pei, X.F., Utsuki, T., Gluck, R. and Acevedo, L.D. (1997). Pharmacological modulation of Alzheimer's bamyloid precursor protein levels in the CSF of rats with forebrain cholinergic system lesions. *Brain Res Mol Brain Res*, 46:161-8
- Herbet, V. (1996). Prooxidant effects of antioxidant vitamins. *Introduction J. Nutr.* 126 (4 Suppl): 1197S-200S.
- Ishola, I. O., Adamson, F. M. and Adeyemi O. O. (2016). Ameliorative effect of kolaviron, a biflavonoid complex from Garcinia kola seeds against scopolamine-induced memory impairment in rats: role of antioxidant defense system. *Metab Brain Dis*
- Ishola, I. O., Tota, S., Adeyemi, O. O., Agbaje, E. O., Narender, T. and Shukla, R. (2013). Protective effect of Cnestis ferruginea and its active constituent on scopolamine-induced memory impairment in mice: a behavioral and biochemical study. *Pharm Biol.*, 51(7):825-835.
- Iwalokun, B. A., Efedede, B. U., Alabi-Sofunde, J. A., Odual, T., Magbagveola, O. A. and Akinwande, A. I. (2006). Hepatoprotective and antioxidant activities of Vernonia amygdalina on acetaminophen-induced hepatic damage in mice. *J. Med. Food.*, 9(4): 524-530.
- Katzman, R., and Saitoh, T. (1991). Advances in Alzheimer's disease. *FASEB J* 5: 278-286.
- Kaur, R., Parveen, S., Mehan, S., Khanna, D. and Kalra, S. (2015). Neuroprotective effect of Ellagic acid against chronically scopolamine induced Alzheimer's Type Memory and Cognitive Dysfunctions: Possible Behavioural and Biochemical Evidences *International Journal of Preventive Medicine Research*. (1): 2, 45-64.
- Khalifa AE. (2004). Pro-oxidant activity of zuclopenthixol in vivo differential effect of the drug on brain oxidative status of scopolamine-treated rats. *Hum Exp Toxicol*; 23 (9):439-45.
- Kumar, A., Dogra, S. and Prakash, A. (2009). Neuroprotective Effects of Centella asiatica against Intracerebroventricular Colchicine Induced Cognitive Impairment and Oxidative Stress. *Int J Alzheimers Dis.*, 9, (7): 21-78.
- Law, A., Gauthier, S. and Quirion, R. (2004). Say NO to Alzheimer's disease: The putative links between nitric oxide and dementia of the Alzheimer's type. *Brain Res Brain Res Rev.*, 35: 73-96
- Lipton, S.A. (2004). Concepts: turning down but not off – neuroprotection requires paradigm shift in drug development. *Nature*, 428, 473.
- Mebratu, A., Yamrot, K., Eyasu, M., Yonas, B., and Kelbesa, U. (2013). Toxic effects of aqueous leaf extracts of Vernonia bipontini Vatke on blood, liver and kidney tissues of mice. *Monona Ethiopian Journal of Science (MEJS)*, 5:15-31.
- Merlo, s., Spampinato, S., Canonico, P. L., Copani, A. and Sortino M.A. (2010). Alzheimer's disease: brain expression

- of a metabolic disorder? Trends in Endocrinology and Metabolism 21: 537–544.
- Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.*, 25; 247(10):3170-5.
- Morris, R. G. (1984). “Development of a water maze procedure for studying spatial learning in the rat.” *J Neurosci Methods.*, 11:47–60.
- Nerurkar, P., Dragull, K. and Tang, C. (2004). In vitro toxicity of kava alkaloid, pipermethystine, in Hep G2 cells compared to kavalactones. *Journal of Toxicological Sciences*, 79:106-111.
- Ogunlade, B., Akuunna, G. G., Fatoba, O. O., Ayeni, O. J., Adegoke, A. and Adelakun, A. (2012). *Vernonia amygdalina* Protects Against Hepatotoxicity in Wistar Rats. *World J Young Researchers*, 2(5)2(5):71
- Ojiako, O.A. and Nwanjo, H.U. (2006). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *Africa Journal of Biotechnology*, 5(18): 1648-1651.
- Onasanwo, S.A., Oyebanjo, O.T., Ajayi, A.M. and Olubori M.A. (2017). Anti-nociceptive and anti-inflammatory potentials of *Vernonia amygdalina* leaf extract via reductions of leucocyte migration and lipid peroxidation (2017). *J Intercult Ethnopharmacol.* 6:2.
- Owolabi, M.A., Jaja, S.I., Oyekeanmi, O.O. and Olatunji O.J. (2008). Evaluation of the antioxidant activity and lipid peroxidation of the leaves of *Vernonia amygdalina*. *J. Compl. Integr. Med.*, 5.
- Rao, A.V. and Balachandran, B. (2002). Role of Oxidative Stress and Antioxidants in Neurodegenerative Diseases. *Nutritional Neuroscience*, 5(5):291-309.
- Pezze, M., Marshall, H. J. and Cassaday H. J. (2017.) Scopolamine Impairs Appetitive But Not Aversive Trace Conditioning: Role of the Medial Prefrontal Cortex. *The Journal of Neuroscience*, 37(26):6289 – 6298.
- Ramirez-Rodriguez, G., Ortiz-Lopez, L., Dominguez-Alonso, A., Benitez-King, G.A. and Kempermann, G. (2011). Chronic treatment with melatonin stimulates dendrite maturation and complexity in adult hippocampal neurogenesis of mice. *J Pineal Res* 50:29-37.
- Rountree, S. D., Chan, W, Pavlik, V. N., Darby, E. J., Siddiqui, S. and Doody, R. S. (2009). Persistent treatment with cholinesterase inhibitors and/or memantine slows clinical progression of Alzheimer disease. *Alzheimers Res Ther.*, 1:7.
- Souza, A.C., Bruning, C.A., Acker, C.I., Neto, J.S. and Nogueira, C.W. (2013). 2-Phenylethynylbutyltellurium enhances learning and memory impaired by scopolamine in mice. *Behav Pharmacol*, 24:249–254.
- Sultana, Z., Takaoka, J. and Koga, T. (2013). Resource value differentially affects fighting success between reproductive and non-reproductive seasons, *J Ethol.*, 31:203–209.
- Susswein, A. J., Katzoff, A., Miller, N. and Hurwitz, I. (2004). Nitric oxide and memory. *Neuroscientist*. 10(2):153-62.
- Tapsell. (2006). Health benefits to herbs and spices: the past, the present and future. *Medicinal Journal of Austria* 1:170-190
- Togo, T., Katsuse, O. and Iseki, E. (2004). Nitric oxide pathways in Alzheimer’s disease and other neurodegenerative dementias. *Neurol Res*, 26: 563–566.
- Tota, S, Nath, C, Najmi, A.K., Shukla, R. and Hanif, K. (2012a.) Inhibition of central angiotensin converting enzyme ameliorates scopolamine induced memory impairment in mice: role of cholinergic neurotransmission, cerebral blood flow and brain energy metabolism. *Behav Brain Res.*, 232:66-76.
- Varshney, R. and Kale, R.K. (1990). Effects of Calmodulin Antagonists on Radiation-induced Lipid Peroxidation in Microsomes. *Int’l j Rad. Biol.*, 58:733-743.
- Walton, N. M., Shin, K., Tajinda, C. L., Heusner, J. H., Kogan, S., Miyake, C. Q., Tamura K 2012. Adult neurogenesis transiently generates oxidative stress. *PLoS One*, 7:e35264
- Wang, W., Li, S., Dong, H., Lv, S. and Tang, Y. (2009). Differential impairment of spatial and non-spatial cognition in a mouse model of brain aging. *Life Sci.*, 85: 127–135.
- Wimo, A., Jönsson, L., Bond, J., Prince, M. and Winblad, B. (2013). The worldwide economic impact of dementia 2010. *Alzheimer disease international. Alzheimers Dement*, 9 (1):1–11.
- Xingxuan H., Huang, Y., Li, B., Gong, C., and Schuchman, E. H. (2010). Deregulation of sphingolipid metabolism in Alzheimer’s disease. *Neurobiology of Aging* 31: 398–408.
- Zaki, N., Efimov, D. and Berenguères, J. (2013). Protein complex detection using interaction reliability assessment and weighted clustering coefficient. *BMC Bioinformatics* 14:163.

Full length Research Article

Mitigation of Diclofenac-Induced Hepatorenal Toxicity by Methanolic Extract of *Cymbopogon citratus*

Adeniyi, T. D.¹, *Moronkeji, A.², Ajala, I.O.³, Oyeleke, A.⁴, Moronkeji, A.I.⁵,
Falana, F.², Ngeri, B.²

¹Department of Medical Laboratory Science, Faculty of Basic Clinical Sciences, University of Ilorin, Kwara State, Nigeria.

²Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Medical Sciences, Ondo State, Nigeria.

³General Medicine Department, Kent and Canterbury Hospital, United Kingdom.

⁴Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Oye-Ekiti, Ekiti State, Nigeria.

⁵Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Elizade University Ilara-Mokin, Ondo State, Nigeria

Summary: Despite the wide range of advantages of non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, their abuse can have a deleterious impact on the hepatorenal system. The phytochemicals in methanolic extracts of *Cymbopogon citratus* have pharmacological properties such as antioxidant and anti-inflammatory properties that can enhance health by preventing and reducing NSAID-induced toxicity. This study evaluated the histopathological and molecular effects of diclofenac-induced hepatorenal toxicity on the expression of TGF β and Nrf2 in the liver and kidneys of adult male Wistar rats treated with methanolic leaf extracts of *C. citratus* (lemon grass). Twenty adult male Wistar rats were randomly divided into four groups. The first group were the unexposed control rats administered with distilled water only, while the test groups (II-IV) were orally administered with diclofenac at a standard dosage of 5mg/kg/BW. The rats in groups III and IV were administered with methanolic extract of *C. citratus* at 100mg/kg/BW and 200mg/kg/BW respectively. After 28 days, the rats were euthanized by cervical dislocation, and the liver and kidneys were histologically processed. mRNA expression of nuclear factor erythroid 2-related factor 2 (Nrf-2) and transforming growth factor beta (TGF- β) was analyzed using the reverse transcription polymerase chain reaction (RT-PCR). The data obtained was statistically analysed using the One-way analysis of variance (ANOVA), and Duncan's multiple range test was employed in comparing categorical variables ($p < 0.05$). The results revealed significant pathological alterations in the histoarchitecture of the livers and kidneys of the untreated diclofenac-exposed rats with the administration of *C. citratus* significantly repressing the expression of TGF- β in the liver and upregulating Nrf-2 levels in the kidneys of the treated rats ($P < 0.05$), while also ameliorating the histological derangement observed in the studied organs. *Cymbopogon citratus* modulates the expression of TGF- β and Nrf-2 in the liver and kidneys of the experimental animals consequently mitigating diclofenac-induced oxidative damage in the studied organs.

Keywords: Diclofenac, drug abuse, lemon grass, medicinal plants, oxidative stress.

*Authors for correspondence: amoronkeji@unimed.edu.ng, Tel: +234-8166785420

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) primarily operate by inhibiting cyclooxygenase (COX) enzymes (Stiller and Hjerdahl 2022). Cyclooxygenase- 1 (COX-1) maintains physiological functions with COX-2 involved in inflammation (Faki and Er 2021). They are widely utilized for their antinociceptive, antipyretic, anti-inflammatory, and analgesic properties (Sriutha *et al.*, 2018; Bindu *et al.*, 2020; Kushner *et al.* 2022). NSAIDs, including diclofenac and ibuprofen, are commonly prescribed cyclooxygenase

inhibitors that have been associated with hepatotoxicity, manifesting as increased liver enzymes, hepatitis, and, in severe cases, acute liver failure. Additionally, they can also cause renal toxicity by directly damaging the renal tubular and glomerular systems, which is primarily caused by impeding prostaglandin synthesis (Sarges *et al.*, 2016; Sriutha, *et al.* 2018; Zoubek *et al.* 2020; Stiller and Hjerdahl, 2022; Boggula *et al.* 2023; Liu and Liu 2024). While NSAIDs effectively alleviate pain and inflammation, they also inhibit COX-1 causing gastrointestinal ulcers and

bleeding. Moreover, NSAIDs have been linked to kidney damage, including acute kidney injury (AKI) and chronic kidney disease (CKD), especially in the elderly (Wongrakpanich et al. 2018; Drożdżal et al. 2021; Dreischulte et al. 2022; Ward et al. 2022). The immunotoxic and hematotoxic effects of NSAIDs have also been documented (Gomaa 2017; Gomaa 2018). The mechanism by which oxidative stress impairs kidney function has been described by Nezu and Suzuki (2020). This mechanism involves the formation of reactive oxygen species (ROS) and electrophiles, which cause damage to the renal tubule epithelial cells and result in the production of damage-associated molecular patterns (DAMPs). In addition, the release of inflammatory cytokines initiates inflammation, which is subsequently mediated by myeloid cells. Furthermore, the conversion of interstitial fibroblast into myofibroblast further promotes kidney fibrosis. The two main syndromes among the various forms of renal failure are CKD and AKI with the likelihood of AKI associated with NSAIDs escalating with prolonged use, particularly in the presence of predisposing factors such as advanced age, diabetes mellitus, and cardiovascular conditions (Ohsaki et al. 2012; Patschan and Müller 2016; Nelson et al. 2019; Kaur et al. 2023; Weng et al. 2024). A cohort study conducted by Lipworth et al. (2016) documented an increase in NSAID use among acute kidney injury survivors. Furthermore, the chronic use of NSAIDs is linked to a higher prevalence of CKD, however, the exact relationship between NSAID use and CKD progression remains somewhat unclear (Wan et al. 2021).

Nuclear factor erythroid 2-related factor 2 or Nrf2 is a transcription factor crucial in inflammation and the regulation of pro-inflammatory genes such as COX-2, that is involved in producing inflammatory mediators, they further play a significant role in cellular defence against toxic and oxidative stress (Ahmed et al. 2017; Nezu and Suzuki 2020; Guerrero-Hue et al. 2021; Ngo and Duennwald, 2022). The activity of Nrf2 is regulated at both transcriptional and post-transcriptional levels, involving protein stability, post-transcriptional modifications, and binding partner availability (Tonelli et al. 2018). Consistent exposure to toxicants can lead to the generation of oxidative stress which is linked to the development of several disorders with studies suggesting that reduction in oxidative stress can inhibit various disease progressions including toxic-induced nephropathy, diabetic nephropathy and hypertension-associated kidney disease (Ratliff et al. 2016; Clarke et al. 2016; Moronkeji and Akinbo 2024).

Transforming growth factor beta (TGF- β) is a pleiotropic cytokine that plays a pivotal role in pro and anti-inflammatory activities. In addition to influencing a variety of biological processes like development, carcinogenesis, immunological responses, wound healing and fibrosis, this regulatory molecule also affects cell differentiation, migration, proliferation, and survival (Sanjabi et al. 2017; Baba et al. 2022). As an anti-inflammatory marker, TGF- β is involved in the biological response to injury and autoimmune diseases, initiating the healing process, which aids wound healing and the resolution of immune responses (Deng et al. 2024).

The increased usage of medicinal plants may be attributed to their acceptability, compatibility, adaptability, and minimal or non-existent negative effects on human

health (Oladeji et al. 2019; Mokhtar et al. 2023). Chronic use of major NSAIDs has been demonstrated to be toxic, and there is evidence that acids exacerbate NSAID-induced injury in patients. In addition to lifestyle modifications, proton pump inhibitors, H₂-receptor antagonists, and antioxidants are used to treat NSAID toxicity (Lazzaroni and Porro 2009; Solomon et al. 2017; Bindu et al., 2020). *Cymbopogon citratus* (lemon grass) is a member of the Poaceae family that is known for its lemony taste and aroma (Oladeji et al. 2019). *C. citratus* is a tall, monocotyledonous aromatic perennial plant with slender sharp-edge green leaves and a pointed apex. It is valued for its anti-inflammatory anti-mutagenicity and antioxidant properties which stem from its various phytoconstituents which include flavonoids, alkaloids and terpenoids and phenolic compounds which are responsible for the plant's therapeutic properties (Umar et al. 2016; Saenthaweesuk et al. 2017; Schabauer et al. 2017; Fahmy et al. 2020; Elekofehinti et al., 2020). The phytochemicals found in *C. citratus* have pharmacological activities that include antioxidant, anti-inflammatory, antiparasitic, anti-obesity, antibacterial, antifungal, antinociceptive and anti-diarrheal qualities. These compounds may improve health by providing a protective and ameliorative effect related to NSAID-induced toxicity (Moronkeji et al. 2024; Okere et al., 2014; Oladeji et al. 2019; Saenthaweesuk et al. 2017; Tarkang et al. 2014). This study evaluates the attenuative potential of methanolic extract of *C. citratus* in NSAID-induced hepatorenal toxicity via modulating the TGF β and Nrf2 signalling pathway.

MATERIALS AND METHODS

Ethical approval: Ethical approval with reference number MNR/V.384/41 was received from the Ministry of Agriculture, Akure while strictly adhering to the established guidelines for the use of experimental animals as stipulated by the research ethics committee which is in alignment with the international Humane Animal Care Standards (Hau and Van Hoosier 2002).

Plant collection, extraction and preparation: Fresh leaves of *Cymbopogon citratus* were harvested from a farm in Ondo town, Ondo state, Nigeria. An expert from the University of Medical Sciences Ondo's (UNIMED) plant biology and biotechnology unit identified the plant and assigned it herbarium identity number 033. The leaves were rinsed in tap water to get rid of any contaminants, shade-dried and further dried in an oven at 60°C and then processed into a powder using an electric blender. The powder was dissolved in methanol and allowed to stand for 24 hours with intermittent shaking ensured. The mixture was first filtered with cheesecloth and the filtrate was filtered again using Whatman filter paper No. 1. The filtrate was then evaporated to dryness using a rotary evaporator under reduced pressure at 40°C. Appropriate concentrations were then diluted in distilled water before being administered throughout the experiment (Moronkeji et al. 2024).

Experimental animals: Twenty adult male Wistar rats weighing 150 and 200 g were purchased from UNIMED's animal holding. The rats were maintained in a clean, well-

ventilated environment and given unrestricted access to water and standard rat pellets produced by New Hope Agriculture and Technology, Nigeria. The experimental animals were acclimatised for two weeks and were randomly divided into four groups consisting of seven rats each ($n = 7$). The first group (Group I) were the unexposed negative control group administered with distilled water only while the second group (Group II) were the diclofenac administered-untreated rats at a dose of 5mg/kg/bw. Rats in groups III and IV received a standard dose of diclofenac, followed by daily treatment with methanolic extract of *C. citratus* at doses of 100 mg/kg BW and 200 mg/kg BW, respectively. The exposure to diclofenac and treatment duration of this administration spanned 28 days and at the end of the experiment, the rats were euthanized and the livers and kidneys were excised and immediately transferred into 10% neutral buffered formalin for histopathological processing for microscopic evaluation. Samples for mRNA expression of TGF- β and Nrf-2 were washed and homogenized in TRIzol for nucleic acid extraction and processed accordingly as described by Molehin *et al.* (2023).

Histological examination: The excised livers and kidneys were fixed in 10% neutral buffered formalin for 48 hours and prepared for paraffin sectioning as described by Adeniyi *et al.*, (2023). Thin sections of approximately 4mm thick were stained with Hematoxylin and Eosin (H&E) and the processed organs were then examined under a light microscope for pathological changes (Moronkeji and Akinbo 2024).

mRNA analysis: The RNA was extracted from the tissues using the Zymo Research Quick-RNA MiniPrep™ kit as directed by the manufacturer and gene expression levels were assessed using the RT-PCR method as outlined by Elekofehinti *et al.* (2020). The RNA purification was carried out from 100-200 mg of tissue using TRIZOL reagent (Inqaba Biotech West Africa Ltd), following the manufacturer's instructions (Invitrogen™, Denmark). The complementary DNA (cDNA) was synthesized using the ProtoScript II First Strand cDNA Synthesis kit (Biolabs, New England), following a 3-step reaction as described by the manufacturer. The PCR for gene expression was conducted using the Luna Mastermix kit (Biolabs, New England) and Taqman kit probes obtained from TibM-01bio (Berlin, Germany), within a thermocycler. The Gel imaging

was performed utilizing an electrophoresis gel imager, with β -actin serving as the reference gene. Standard gel electrophoresis was employed as the technique for analyzing the quality and yield of DNA products from the reaction. The cDNA primers utilized were procured from Inqaba Biotech (Hatfield, South Africa). The specific primers employed for PCR are as follows:

TGF- β :

forward (5'-ATACGCCTGAGTGGCTGTCT-3')
reverse (5'-TGGGACTGATCCCATTGATT-3').

NRF2:

forward (5'-GGGGAACAGAACAGGAAACA-3')
reverse (5'-CCGTAATGCACGGCTAAGTT-3').

β -actin:

forward (5'-CCCGCGAGTACAACCTTCT-3')
reverse (5'-CGTCATCCATGGCGAACT-3').

Statistical analysis: The results were pooled and expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) followed by Duncan's multiple tests for post-hoc analysis (DMRT) was employed to analyze the results. The statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 17.0, with a 95% confidence interval ($p < 0.05$) used to determine the significance level.

RESULTS

Histopathological findings: Histopathological evaluation of the H&E-stained sections of the liver of the unexposed control rats appeared normal, with typical hepatocytes with a non-congested central vein or infiltrated sinusoid (Figure 1a). However, the liver of the diclofenac-exposed untreated rats had varying degrees of cytopathic lesions, evidenced by congested venules, and vacuolated hepatocytes with mildly congested sinusoids (Figure 1b). The liver of the *C. citratus* treated rats at 100mg/kg/bw had mildly congested central veins with normal appearing hepatocytes and sinusoids devoid of inflammation and congestion (Figure 1c). The rats treated with *C. citratus* at 200mg/kg/bw had a near normal histoarchitecture with the central vein devoid of congestion with uncongested sinusoids and normal appearing hepatocytes (Figure 1d).

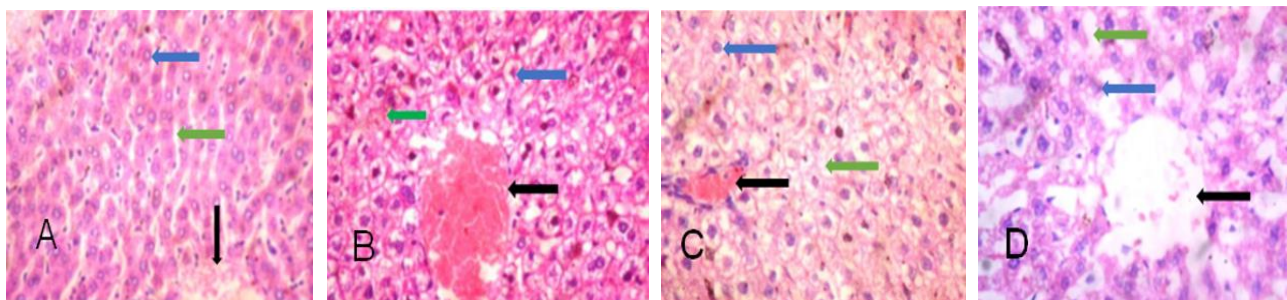


Plate 1

H and E-stained section of the liver of rats across the various groups. **A.** Central vein (black arrow), sinusoids (green arrow) hepatocytes (blue arrow) **B.** Congested venule (black arrow), vacuolated and hepatocytic necrosis (blue arrow), congested sinusoids (green arrow). **C.** Mildly congested central vein (black arrow), hepatocytes (blue arrow), sinusoids (green arrow). **D.** non-congested central vein (black arrow), hepatocytes (blue arrow), sinusoids (green arrow)

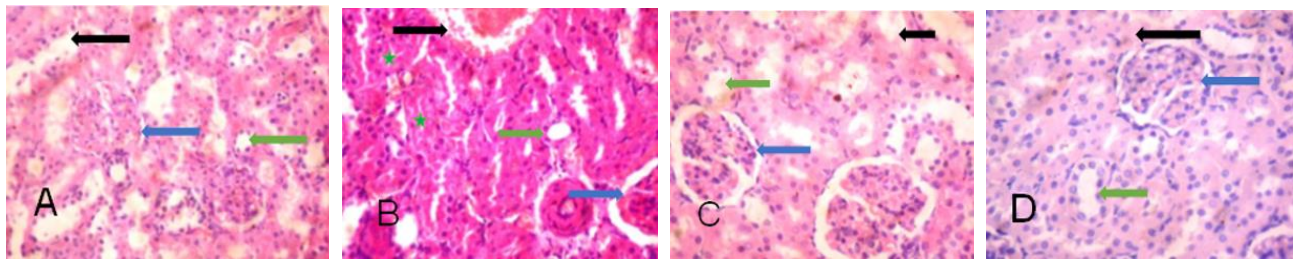


Plate 2.

H and E-stained sections of the kidneys of rats across the various groups. **A** glomerulus (blue arrow), renal tubules (green arrow) interstitial spaces (black arrow). **B**. Glomerulus (blue arrow), renal tubule (green arrow), renal tubule with reduced luminal space (starred green), congested interstitium (black arrow). **C**. glomerulus (blue arrow), renal tubule (green arrow), interstitial space (black arrow). **D**. Glomerulus (blue arrow) renal tubule (green arrow) non-congestion or non-inflamed interstitium (black arrow).

The kidneys of the unexposed control rats were normal and devoid of any pathological lesion with the glomerulus, renal tubules and interstitial spaces appearing normal (Figure 2a). Diclofenac-exposed untreated rats had congested interstitial spaces with mild tubular degeneration (Figure 2b). The treatment with 100mg/kg/bw of *C. citratus* showed better recovery compared to the diclofenac-exposed untreated rats showing normal glomeruli, and renal tubules with non-congested or non-inflamed interstitium (Figure 2c). The kidney sections of rats administered with 200mg/kg/BW of *C. citratus* were devoid of cytopathic lesions demonstrating a near normal histoarchitecture as the unexposed negative control rats (Figure 2d).

mRNA expression studies: Figure 3 shows the relative expression levels of TGF- β and Nrf-2 in the liver and kidneys of the NSAIDs-exposed untreated rats relative to the control and other treatment groups. TGF- β being a pleiotropic cytokine was significantly upregulated in the diclofenac-exposed untreated rats when compared to the control and other treatment groups indicating the induction of diclofenac-induced oxidative stress in the liver. However, the treatment with *C. citratus* mitigated the toxicity by repressing the expression levels of TGF- β with treatments at doses of 100mg/kg/bw and 200mg/kg/bw. Furthermore, comparative analysis across the 100mg/kg/bw and 200mg/kg/bw treatments with extracts of *C. citratus* was statistically insignificant (Figure 3a). The Nrf-2 expression pattern in the diclofenac-exposed untreated rats was downregulated when compared to other groups while treatment with *C. citratus* at a dosage of 100mg/kg/bw and 200mg/kg/bw significantly elevated the expression levels of Nrf-2 relative to the diclofenac-exposed untreated rats indicating a reduction in the diclofenac induced oxidative stress in the kidneys of the treated rats (Figure 3b).

DISCUSSION

Prolonged exposure to NSAIDs has been shown to cause hepatorenal disorders, and inappropriate use of diclofenac can cause damage to the liver and kidneys (Sriutha et al. 2018; Delungahawatta et al. 2023; Boggula et al., 2023; Liu and Liu 2024). Globally, over 850 million people suffer from kidney disease. Consequently, understanding the complex molecular pathology of kidney disease is crucial for creating novel treatments for renal diseases (Jager et al. 2019; Nezu and Suzuki 2020). Studies have demonstrated that the constituents of medicinal plants possess a variety of activity against various disorders and diseases.

Additionally, these compounds have been shown to have analgesic, antioxidant, and immunomodulatory properties (Schabauer et al., 2017; Adeniyi et al., 2024; Moronkeji et al. 2024). By comparing the experimental animals treated with methanolic extracts of *C. citratus* to the untreated diclofenac-exposed rats, the histopathological examination in this study revealed remediation of cytopathic lesions associated with diclofenac-induced hepatorenal toxicity. This further provides insights into the protective effects of *Cymbopogon citratus* against diclofenac-induced hepatorenal toxicity. The findings in this study are consistent with previous studies showing that diclofenac caused notable structural alterations in the liver and kidneys of the experimental animals.

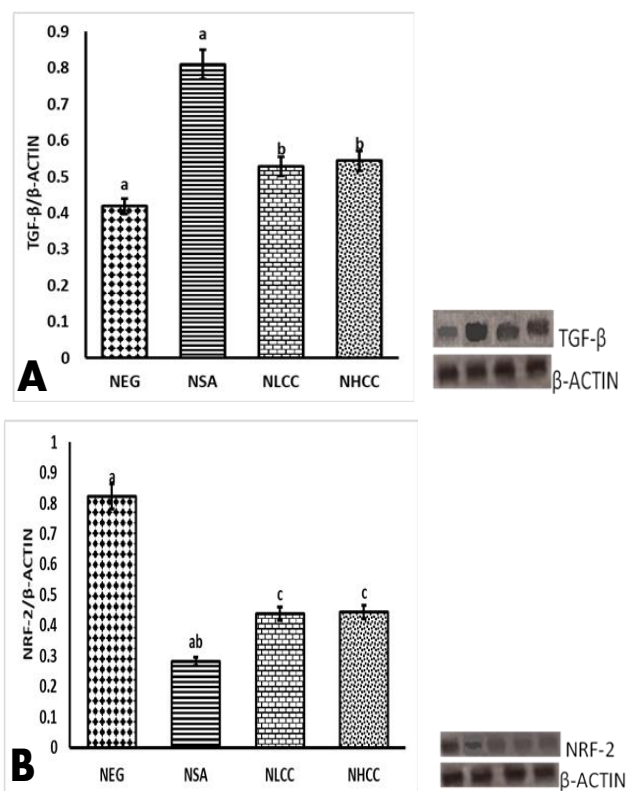


Figure 3:

mRNA expression pattern of markers in the liver and kidney **a:** expression pattern of TGF- β in the liver. **b:** expression pattern of Nrf-2 in the kidney.

Keys: Neg; unexposed negative control; NSA; NSAIDs (diclofenac) exposed untreated rats; NLCC; NSAIDs (diclofenac) exposed low *C. citratus* treatment at 100mg/kg/bw; NHCC: NSAIDs (diclofenac) exposed high *C. citratus* treatment at 200mg/kg/bw.

Cytopathic lesions included hepatocytic vacuolation, inflamed and congested sinusoids combined with congested venules, periglomerular inflammation, and dilated interstitial spaces were evident in the diclofenac exposed untreated rats (Bindu *et al.* 2020; Delungahawatta *et al.* 2023; Adel *et al.* 2024). Furthermore, earlier studies by Sriuttha *et al.* (2018) had shown that NSAIDs like diclofenac had greater rates of hepatotoxic evidence than other NSAIDs like celecoxib and etoricoxib. However, treatment with *C. citratus* showed a dose-dependent improvement in liver histology, with treatments at 100 and 200 mg/kg/bw considerably ameliorating the lesions induced by diclofenac. Following treatment with 100 mg/kg/BW of *C. citratus*, liver sections exhibited uncongested sinusoids and slightly congested central veins with normal-appearing hepatocytes with better amelioration observed at treatment with 200 mg/kg/BW further corroborating the reports of Saenthaweesuk *et al.* (2017) who demonstrated the hepatoprotective effects of *C. citratus* extract against paracetamol-induced hepatotoxicity. Additional studies have also shown that *C. citratus* protects against NSAID-induced hepatotoxicity, owing to its ability to modulate TNF- α and IL-10 levels (Moronkeji *et al.*, 2024). Moreover, studies on *C. citratus* have shown significant antioxidant, hepatorenal protection, and anti-mutagenic efficacy against hepatorenal damage generated by carbon tetrachloride (CCl₄) (Fahmy *et al.* 2020; Molehin *et al.* 2023). Lesions typical of tubular degeneration coupled with congested interstitium were seen in the renal histoarchitecture of the untreated rats exposed to diclofenac. These findings are consistent with those of Bindu *et al.* (2020), who reported similar renal damage in NSAID-induced nephrotoxicity characterised by tubular injury and vascular congestion. Jarrar *et al.* (2019) also documented that NSAIDs also caused histological changes including sinusoidal dilatation, apoptotic hepatocytes, renal tubule hydropic degeneration, glomerular atrophy, and renal cell karyopyknosis that could affect organ function. In contrast to the diclofenac exposed untreated rats, treatment with *C. citratus* led to better recovery of the kidney as glomeruli and renal tubules appeared normal with interstitium devoid of inflammation and congestion, exhibiting near-normal histoarchitecture further suggesting a nephroprotective effect of *C. citratus*, in the context of NSAID-induced nephrotoxicity. The results of this investigation are in line with the general anti-inflammatory and antioxidant properties of *C. citratus* that have been reported by Oladeji *et al.* (2019) and Moronkeji *et al.* (2024). Studies have shown a correlation between NSAID usage and glomerulonephritis and acute kidney injury. NSAID use in the conventional sense is linked to a higher relative risk of nephrotic syndrome and a decrease in kidney haemodynamic functions, such as sodium excretion (Rivosecchi *et al.* 2016; Bakhriansyah *et al.* 2019). Nephrotic syndrome hypersensitivity mechanisms involving NSAIDs are caused by inhibiting prostaglandin synthesis from COX to lipoxygenase pathways, or by releasing lymphokines that raise leukotriene production and activate T-helper lymphocytes, which in turn affect glomerular permeability (Vega *et al.* 2012). The mRNA studies assessed the TGF- β and Nrf-2 expression pattern in NSAID-induced hepatorenal toxicity, offering an additional

understanding of the molecular mechanism underlying the protective effects of *C. citratus*. TGF- β expression in the liver was upregulated in the diclofenac-exposed untreated rats, while treatments with *C. citratus* at doses of 100 and 200 mg/kg/bw repressed TGF- β expression levels. As a pleiotropic cytokine, TGF- β is essential for both pro- and anti-inflammatory processes (Sanjabi *et al.* 2017; Deng *et al.* 2024). As previously reported by Laskin *et al.* (2011), its increase in the diclofenac-exposed untreated rats suggests an inflammatory response and an effort at tissue repair via activation of hepatic stellate cells and stimulation of extracellular matrix protein synthesis. Studies have revealed that defects in TGF- β signalling, particularly in immune cells, tissue fibroblasts, and epithelial cells disrupt immunological tolerance, encourage inflammation, and are responsible for the aetiology of fibrosis and cancer which can contribute to the resistance of these conditions to therapy (Massagué and Sheppard, 2023; Deng *et al.* 2024). In the *C. citratus* treatment groups, TGF- β was found to be moderately expressed compared to the unexposed control and the diclofenac-exposed untreated rats. This suggests that TGF- β may contribute to reducing inflammatory responses while still facilitating tissue repair, further highlighting its potential to maintain an equilibrium in the inflammatory response and potentially prevent excessive fibrosis while promoting tissue repair. This is consistent with the report of Massagué and Sheppard (2023) who documented the intricate function of TGF- β in tissue homeostasis and disease. Furthermore, reports by Laskin *et al.* (2011) and Gauthier *et al.* (2023) reported that TGF- β suppressed the production of pro-inflammatory cytokines by activating the transcriptional coactivator SMAD3 and suppressing the activity of pro-inflammatory transcription factors NF- κ B, STAT1, and AP-1. The findings in this study demonstrated a decrease in cytopathic lesions following treatment with *C. citratus* at doses of 100 mg/kg BW and 200 mg/kg BW along with moderately expressed TGF- β expression, further indicating the role in inflammation and tissue repair.

Nuclear factor E2-related factor 2 (Nrf2) is an essential transcription factor of cellular defense mechanism against xenobiotics and oxidative stress. It regulates the activation of several cytoprotective genes at both the basal and stress-induced levels (Tonelli *et al.*, 2018). This study details the function of *C. citratus* in reducing oxidative damage associated with diclofenac-induced renal toxicity. This is achieved by upregulating the expression of genes related to cryoprotection, which helps mitigate the histoarchitectural distortion observed in the examined organs. The findings in this study are consistent with the reports of Nezu and Suzuki (2020) highlighted the role of Nrf2 in renal toxicity using murine model to emphasize the significance of Nrf2 in renal damage. Comparing the unexposed control group to the diclofenac-exposed untreated rats, it was observed that Nrf2 was significantly repressed while Nrf-2 was upregulated in rats treated with *C. citratus* at the administered doses. This finding is particularly significant when considered in light of a previous study by Ahmed *et al.* (2017) who described Nrf-2 as a crucial transcription factor in regulating cellular defense against oxidative stress and inflammation. Although, clinical research is still ongoing to develop therapies to treat kidney diseases with Nrf2 activators that block Keap1 (Kelch-like ECH-

associated protein 1), which represses Nrf2 in the absence of oxidative stress (Pergola *et al.* 2011; de Zeeuw *et al.* 2013; Nezu, 2017; Yamamoto *et al.*, 2018; Ito *et al.*, 2020). The repression of Nrf2 observed in the diclofenac-exposed untreated rats indicates compromised antioxidant defences, which is consistent with the known mechanisms of NSAID-induced toxicity involving oxidative stress (Stiller and Hjendahl 2022; Delungahawatta *et al.* 2023). Studies using Knockout mice models have highlighted the protective roles of Nrf2 in various disease models such as respiratory disease (Cho *et al.* 2013; Eba *et al.* 2013; Sussan *et al.* 2015; Nagashima *et al.* 2019) cardiac disease (Katsumata *et al.* 2014), neurological disease (Linker *et al.* 2011; Uruno *et al.* 2020), diabetes mellitus (Yagishita *et al.* 2014; Yagishita *et al.* 2017; Yagishita *et al.* 2019), inflammation (Itoh *et al.* 2004; Maicas *et al.* 2011), liver disease (Taguchi *et al.* 2019; Okada *et al.* 2013) and sensory organ diseases (Knatko *et al.* 2015; Xu *et al.* 2014). Furthermore, studies indicate that the loss of Nrf2 exacerbates tissue damage and fibrosis in kidney disease models, including toxic injury, ureteral obstruction and podocyte injury (Tanaka *et al.* 2008; Tan *et al.* 2016). These findings suggest that renal damage may be prevented by quenching oxidative stress through Nrf2 activation in tubules, which may be a potential cause of kidney disease. According to reports, systemic Nrf2 activation in Keap1 hypomorphic mutant (Keap1 knockdown; Keap1-KD) significantly attenuates 8-hydroxydeoxyguanosine (8-OHdG) mitigating kidney injury progression relative to wild-type mice (Nezu *et al.* 2017). Additionally, Nrf2 functions as an integrator, promoting the expression of genes encoding antioxidant-producing enzymes like glutathione and NADPH and lowering pro-oxidants such as heme and quinonoids further protecting cells from oxidative damage (Yamamoto *et al.*, 2018; Nezu, Suzuki, and Yamamoto 2017). Studies and clinical trials involving chemical compounds that activate Nrf2 in rats exposed to a toxic injury model have documented the beneficial role of Nrf2 activation in treating kidney disease. Compounds such as curcumin, dimethyl fumarate, farrerol, and sulforaphane have been shown to improve the outcome of toxic injury-induced kidney disease (Zhao *et al.* 2016; Wu *et al.* 2017; Li *et al.* 2019; Jin *et al.* 2020; Ma *et al.* 2019). In this study, the upregulation of Nrf-2 in the *C. citratus* treatment groups at 100mg/kg/bw and 200mg/kg/bw further suggests enhanced antioxidant mechanisms, potentially contributing to the protective effects against diclofenac-induced toxicity. Further evidence that maximal protection can be obtained at less toxic doses comes from our observation that the experimental animal treated with *C. citratus* at a lower dosage of 100mg/kg/bw experienced the same immunomodulatory effect on Nrf-2 as did the animal treated with a higher dose of 200mg/kg/bw. A possible mechanism and antioxidant properties of *C. citratus* for the observed nephroprotective effects as observed in this study further agree with the reports of Oladeji *et al.* (2019). Previous reports by Ma, (2013) and Guerrero-Hue *et al.* (2021) reported the protective function of Nrf2 in cellular defence against toxic and oxidative stress and renal disease.

In conclusion, *Cymbopogon citratus* demonstrated significant hepatorenal protective properties by reducing the damage induced by diclofenac. Studies on mRNA expression of the plant have also supported the anti-

inflammatory potential of *C. citratus* by repressing the expression of TGF- β while upregulating Nrf-2 levels in the treated rats. Furthermore, the methanolic extracts of *C. citratus* can serve as TGF- β and Nrf2 activators and can be harnessed for proper management in hepatorenal disease treatment. This study further establishes the cytoprotective role of TGF- β and Nrf2 pathways and unveils the regulatory mechanism of their activities in response to diclofenac-induced hepatorenal toxicity. It is further suggested that caution needs to be exercised when NSAIDs are to be administered which should be limited to the lowest therapeutic doses, to prevent its harmful effect.

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REFERENCES

- Adel EA., Kamal, H.I., Magdy, M.A., Gamal T., Hamid, L., Ragab, S.F.M., Khodier S.A, Mohamed, A., Kamel, M.M., El-shahawy, A.A., Yehia, Y.D.A. (2024). Review on diclofenac toxicities in different organs. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*, 42(1), 10-24.
- Adeniyi, T. D., Moronkeji A., Omokehinde O.A., Firdaws O., and Mofesola, A. (2024). Evaluation of the Combined Extract of Rauwolfia vomitoria and Aframomum melegueta on the Kidney and Liver of Adult Male Wistar Rats. *Sokoto Journal of Medical Laboratory Science*. 9(2): 203–12.
- Adeniyi, T. D., Moronkeji, A., & Ralph-Okhiria, O. F. (2023). Ascorbate and alpha-tocopherol mitigate toxic pathological changes in adult wistar rats exposed to cypermethrin. *Folia Medica Indonesiana*, 59(4), 387-395.
- Ahmed, S. M. U., Luo, L., Namani, A., Wang, X. J., & Tang, X. (2017). Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochimica et Biophysica Acta (BBA)-Molecular basis of disease*, 1863(2), 585-597.
- Arfè, A., Scotti, L., Varas-Lorenzo, C., Nicotra, F., Zambon, A., Kollhorst, B., Schink, T., Garbe, E., Herings, R., Straatman, H., Schade, R., Villa, M., Lucchi, S., Valkhoff, V., Romio, S., Thiessard, F., Schuemie, M., Pariente, A., Sturkenboom, M & Corrao, G. (2016). Non-steroidal anti-inflammatory drugs and risk of heart failure in four European countries: nested case-control study. *BMJ*, 354, i4857.
- Baba, A. B., Rah, B., Bhat, G. R., Mushtaq, I., Parveen, S., Hassan, R., Hameed, Z.M., & Afroze, D. (2022). Transforming growth factor-beta (TGF- β) signaling in cancer-A betrayal within. *Frontiers in pharmacology*, 13, 791272.
- Bakhriansyah, M., Souverein, P. C., van den Hoogen, M. W., De Boer, A., & Klungel, O. H. (2019). Risk of nephrotic syndrome for non-steroidal anti-inflammatory drug users. *Clinical Journal of the American Society of Nephrology*, 14(9), 1355-1362.
- Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical pharmacology*, 180, 114147.
- Boggula, N., Tadikonda, R. R., & Pasam, S. Nephrotoxicity and hepatotoxicity associated with non-steroidal anti-inflammatory drugs. *International Journal of Research in Pharmacy and Pharmaceutical Sciences* 8(4): 1–9.

- Cho, H. Y., Gladwell, W., Yamamoto, M., & Kleeberger, S. R. (2013). Exacerbated airway toxicity of environmental oxidant ozone in mice deficient in Nrf2. *Oxidative medicine and cellular longevity*, 2013(1), 254069.
- Clarke, J. L., Murray, J. B., Park, B. K., & Copple, I. M. (2016). Roles of Nrf2 in drug and chemical toxicity. *Current Opinion in Toxicology*, 1, 104-110.
- De Zeeuw, D., Akizawa, T., Audhya, P., Bakris, G.L., Chin, M., Christ-Schmidt, H., Goldsberry, A., Houser, M., Krauth, M., Lambers Heerspink, H.J. and McMurray, J.J.(2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *New England Journal of Medicine*, 369(26), 2492-2503.
- Delungahawatta, T., Pokharel, A., Paz, R., & Haas, C. J. (2023). Topical Diclofenac-Induced Hepatotoxicity. *Journal of Community Hospital Internal Medicine Perspectives*, 13(3), 108.
- Deng, Z., Fan, T., Xiao, C., Tian, H., Zheng, Y., Li, C., & He, J. (2024). TGF- β signaling in health, disease, and therapeutics. *Signal transduction and targeted therapy*, 9(1), 61.
- Dreischulte, T., Shahid, F., Muth, C., Schmiedl, S., & Haefeli, W. E. (2022). Prescribing cascades: how to detect them, prevent them, and use them appropriately. *Deutsches ärzteblatt international*, 119(44), 745-52.
- Drożdżal, S., Lechowicz, K., Szostak, B., Rosik, J., Kotfis, K., Machoy-Mokrzyńska, A., Białecka, M., Ciechanowski, K., & Gawrońska-Szklarz, B. (2021). Kidney damage from nonsteroidal anti-inflammatory drugs—Myth or truth? Review of selected literature. *Pharmacology research & perspectives*, 9(4), e00817.
- Eba, S., Hoshikawa, Y., Moriguchi, T., Mitsuishi, Y., Satoh, H., Ishida, K., Watanabe, T., Shimizu, T., Shimokawa, H., Okada, Y. and Yamamoto, M., (2013). The Nuclear Factor Erythroid 2–Related Factor 2 Activator Oltipraz Attenuates Chronic Hypoxia-Induced Cardiopulmonary Alterations in Mice. *American journal of respiratory cell and molecular biology*, 49(2), 324-333.
- Elekofehinti, O. O., Onunkun, A. T., & Olaleye, T. M. (2020). *Cymbopogon citratus* (DC.) Stapf mitigates ER-stress induced by streptozotocin in rats via down-regulation of GRP78 and up-regulation of Nrf2 signaling. *Journal of Ethnopharmacology*, 262, 113130.
- Fahmy, M. A., Aly, F. A. E., Hassan, E. M., Farghaly, A. A., Hassan, E. E., & Sâmea, N. S. A. (2020). *Cymbopogon citratus* essential oil alleviates the genotoxicity and oxidative stress of carbon tetrachloride in mice. *Comunicata Scientiae*, 11, 1-8.
- Faki, Y., & Er, A. (2021). Different chemical structures and physiological/pathological roles of cyclooxygenases. *Rambam Maimonides medical journal*, 12(1), 1–13.
- Gauthier, T., Yao, C., Dowdy, T., Jin, W., Lim, Y. J., Patiño, L. C., Liu N, Ohlemacher, S.I., Bynum, A., Kazmi, R., Bewley, C.A. (2023). TGF- β uncouples glycolysis and inflammation in macrophages and controls survival during sepsis. *Science signaling*, 16(797), eade0385.
- Gomaa, S. (2017). Immunomodulatory and hematological effects induced by diclofenac, ibuprofen or paracetamol toxicity in Swiss albino mice. *European Journal of Biological Research*, 7(4), 348-359.
- Gomaa, S. (2018). Adverse effects induced by diclofenac, ibuprofen, and paracetamol toxicity on immunological and biochemical parameters in Swiss albino mice. *The journal of basic and applied zoology*, 79, 1-9.
- Guerrero-Hue, M., Rayego-Mateos, S., Vázquez-Carballo, C., Palomino-Antolín, A., García-Caballero, C., Opazo-Rios, L., Morgado-Pascual, J.L., Herencia, C., Mas, S., Ortiz, A. and Rubio-Navarro, A. (2020). Protective role of Nrf2 in renal disease. *Antioxidants*, 10(1), 39.
- Hau, J., and Van-Hoosier, G. (2002). Handbook of Laboratory Animal Science, Second Edition: Essential Principles and Practices Handbook of Laboratory Animal Science, Second Edition: Essential Principles and Practices. Second. ed. Jr. Jann Hau and Gerald L. Van Hoosier. USA: CRC Press.
- Ito, M., Tanaka, T., & Nangaku, M. (2020). Nuclear factor erythroid 2-related factor 2 as a treatment target of kidney diseases. *Current Opinion in Nephrology and Hypertension*, 29(1), 128-135.
- Itoh, K., Mochizuki, M., Ishii, Y., Ishii, T., Shibata, T., Kawamoto, Y., Kelly, V., Sekizawa, K., Uchida, K. and Yamamoto, M. (2004). Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy- Δ 12, 14-prostaglandin J2. *Molecular and cellular biology*. 24(1): 36–45.
- Jager, K. J., Kovesdy, C., Langham, R., Rosenberg, M., Jha, V., & Zoccali, C. (2019). A single number for advocacy and communication—worldwide more than 850 million individuals have kidney diseases. *Kidney International* (2019) 96, 1048–1050
- Jarrar, Y. B., Jarrar, Q., & Abu-Shalhooob, M. (2019). Effects of nonsteroidal anti-inflammatory drugs on the expression of arachidonic acid-metabolizing Cyp450 genes in mouse hearts, kidneys and livers. *Prostaglandins & Other Lipid Mediators*, 141, 14-21.
- Jin, F., Chen, X., Yan, H., Xu, Z., Yang, B., Luo, P., & He, Q. (2020). Bisdemethoxycurcumin attenuates cisplatin-induced renal injury through anti-apoptosis, anti-oxidant and anti-inflammatory. *European Journal of Pharmacology*, 874, 173026.
- Katsumata, Y., Shinmura, K., Sugiura, Y., Tohyama, S., Matsushashi, T., Ito, H., Yan, X., Ito, K., Yuasa, S., Ieda, M. and Urade, Y. (2014). Endogenous prostaglandin D2 and its metabolites protect the heart against ischemia-reperfusion injury by activating Nrf2. *Hypertension*, 63(1), 80-87.
- Kaur, A., Sharma, G. S., & Kumbala, D. R. (2023). Acute kidney injury in diabetic patients: A narrative review. *Medicine*, 102(21), e33888.
- Knatko, E.V., Ibbotson, S.H., Zhang, Y., Higgins, M., Fahey, J.W., Talalay, P., Dawe, R.S., Ferguson, J., Huang, J.T.J., Clarke, R. and Zheng, S. (2015). Nrf2 activation protects against solar-simulated ultraviolet radiation in mice and humans. *Cancer Prevention Research*, 8(6), 475-486.
- Kushner, P., McCarberg, B.H., Grange, L., Kolosov, A., Haveric, A.L., Zucal, V., Petruschke, R. and Bissonnette, S. (2022). The use of non-steroidal anti-inflammatory drugs (NSAIDs) in COVID-19. *NPJ Primary Care Respiratory Medicine*, 32(1), 35.
- Laskin, D. L., Sunil, V. R., Gardner, C. R., & Laskin, J. D. (2011). Macrophages and tissue injury: agents of defense or destruction? Annual review of pharmacology and toxicology, 51(1), 267-288.
- Lazzaroni, M., and Porro, G.B. (2009). Management of NSAID-Induced Gastrointestinal Toxicity: Focus on Proton Pump Inhibitors. *Drugs*, 69(1): 51–69.
- Li, Y., Zhang, J., Liu, H., Yuan, J., Yin, Y., Wang, T., ... & Guo, Z. (2019). Curcumin ameliorates glyoxylate-induced calcium oxalate deposition and renal injuries in mice. *Phytomedicine*, 61, 152861.
- Linker, R.A., Lee, D.H., Ryan, S., van Dam, A.M., Conrad, R., Bista, P., Zeng, W., Hronowsky, X., Buko, A., Chollate, S. and Ellrichmann, G. (2011). Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain*, 134(3), 678-692.

- Lipworth, L., Abdel-Kader, K., Morse, J., Stewart, T.G., Kabagambe, E.K., Parr, S.K., Birdwell, K.A., Matheny, M.E., Hung, A.M., Blot, W.J. and Ikizler, T.A. (2016). High prevalence of non-steroidal anti-inflammatory drug use among acute kidney injury survivors in the southern community cohort study. *BMC nephrology*, 17, 1-9.
- Liu, Y., & Liu, M. W. (2024). A unusual presentation of liver failure caused by Ibuprofen-sustained release capsules: A case report. *Medicine*, 103(4), e36997.
- Ma, N., Wei, W., Fan, X., & Ci, X. (2019). Farrerol attenuates cisplatin-induced nephrotoxicity by inhibiting the reactive oxygen species-mediated oxidation, inflammation, and apoptotic signaling pathways. *Frontiers in physiology*, 10, 1419.
- Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annual review of pharmacology and toxicology*, 53(1), 401-426.
- Maicas Blasco, N., Ferrándiz, M. L., Brines, R., Ibáñez, L., Cuadrado, A., Koenders, M. I., ... & Alcaraz, M. J. (2011). Deficiency of Nrf2 accelerates the effector phase of arthritis and aggravates joint disease. *Antioxidants and Redox Signaling* 15(4): 889–901.
- Massagué, J., & Sheppard, D. (2023). TGF- β signaling in health and disease. *Cell*, 186(19), 4007-4037.
- Mokhtar, L. M., Salim, I. A., Alotaibi, S. N., Awaji, E. A., Alotaibi, M. M., & Doman, A. O. (2023). Phytochemical Screening and Antimicrobial Activity of Methanolic Extract of *Cymbopogon schoenanthus* (L.) (azkhar) Collected from Afif City, Saudi Arabia. *Life*, 13(7), 1451.
- Molehin, O., Elekofehinti, O. O., Oyeyemi, A. O., & Olubaju, O. O. (2023). Molecular Investigation of the Ameliorative Potentials of *Cymbopogon citratus* Leaves Extract in Carbon tetrachloride-Induced Hepatotoxicity in Rats. *The Journal of Pharmacology and Experimental Therapeutics*. 385.
- Moronkeji, A., & Akinbo, F. O. (2024). Genotoxic Response and Histological Alterations in Rat Lungs Exposed to Gasoline Generator Exhaust. Sultan Qaboos University *Journal for Science* [SQUJS]. 29(1): 15–27.
- Moronkeji, A., Adeniyi, T. D., Oyeleke, A., Igunbor, C. M., Ayeni, J. O., Adelabi, T. O., & Moronkeji, A.I. (2024). *Cymbopogon citratus* : A Modulator of TNF- α and IL-10 in NSAID-Induced Hepatorenal Toxicity in Adult Wistar Rats. *Bulletin of Pharmaceutical Sciences Assiut University* 47(2): 1251–62.
- Moronkeji, A., Temidayo, A., Benita, A., & Latifat, O. (2024). Methanolic leaf extract of *Vernonia amygdalina* mitigates cadmium-induced oxidative stress in the liver and kidney of adult male Wistar rats. *Egyptian Journal of Basic and Applied Sciences*, 11(1), 469-478.
- Nagashima, R., Kosai, H., Masuo, M., Izumiyama, K., Noshikawaji, T., Morimoto, M., ... & Tanaka, N. (2019). Nrf2 suppresses allergic lung inflammation by attenuating the type 2 innate lymphoid cell response. *The Journal of Immunology*, 202(5), 1331-1339.
- Nelson, D. A., Marks, E. S., Deuster, P. A., O'Connor, F. G., & Kurina, L. M. (2019). Association of nonsteroidal anti-inflammatory drug prescriptions with kidney disease among active young and middle-aged adults. *JAMA network open*, 2(2), e187896-e187896.
- Nezu, M., & Suzuki, N. (2020). Roles of Nrf2 in protecting the kidney from oxidative damage. *International Journal of Molecular Sciences*, 21(8), 2951.
- Nezu, M., Souma, T., Yu, L., Suzuki, T., Saigusa, D., Ito, S., Suzuki, N. and Yamamoto, M. (2017). Transcription factor Nrf2 hyperactivation in early-phase renal ischemia-reperfusion injury prevents tubular damage progression. *Kidney International*, 91(2), 387-401.
- Nezu, M., Suzuki, N., & Yamamoto, M. (2017). Targeting the KEAP1-NRF2 system to prevent kidney disease progression. *American journal of nephrology*, 45(6), 473-483.
- Ngo, V., & Duennwald, M. L. (2022). Nrf2 and oxidative stress: A general overview of mechanisms and implications in human disease. *Antioxidants*, 11(12), 2345.
- Ohsaki, Y., O'Connor, P., Mori, T., Ryan, R.P., Dickinson, B.C., Chang, C.J., Lu, Y., Ito, S. and Cowley Jr, A.W., (2012). Increase of sodium delivery stimulates the mitochondrial respiratory chain H₂O₂ production in rat renal medullary thick ascending limb. *American Journal of Physiology-Renal Physiology*, 302(1), 95-102.
- Okada, K., Warabi, E., Sugimoto, H., Horie, M., Gotoh, N., Tokushige, K., Hashimoto, E., Utsunomiya, H., Takahashi, H., Ishii, T. and Yamamoto, M. (2013). Deletion of Nrf2 leads to rapid progression of steatohepatitis in mice fed atherogenic plus high-fat diet. *Journal of Gastroenterology*, 48, 620-632.
- Okere, S. O., Sangodele, J. O., Ogunwole, E., Adams, M. D., & Shafe, M. O. (2014). Antiplasmodial activity of aqueous leaf extract of *Cymbopogon citratus* against *Plasmodium falciparum* infected rats. *American Journal of Biomedical and Life Sciences*, 2(3), 60-64.
- Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., & Odelade, K. A. (2019). Phytochemistry and pharmacological activities of *Cymbopogon citratus* : A review. *Scientific African*, 6, e00137.
- Patschan, D., & Müller, G. A. (2016). Acute kidney injury in diabetes mellitus. *International journal of nephrology*, 2016(1), 6232909.
- Pergola, P.E., Raskin, P., Toto, R.D., Meyer, C.J., Huff, J.W., Grossman, E.B., Krauth, M., Ruiz, S., Audhya, P., Christ-Schmidt, H. and Wittes, J. (2011). Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *New England Journal of Medicine*, 365(4), 327-336.
- Ratliff, B. B., Abdulmahdi, W., Pawar, R., & Wolin, M. S. (2016). Oxidant mechanisms in renal injury and disease. *Antioxidants & redox signaling*, 25(3), 119-146.
- Rivosecchi, R.M., Kellum, J.A., Dasta, J.F., Armahizer, M.J., Bolesta, S., Buckley, M.S., Dzierba, A.L., Frazee, E.N., Johnson, H.J., Kim, C. and Murugan, R. (2016). Drug class combination-associated acute kidney injury. *Annals of Pharmacotherapy*, 50(11), 953-972.
- Saenthaweesuk, S., Munkong, N., Parklak, W., Thaeomom, A., Chaisakul, J., & Somporn, N. (2017). Hepatoprotective and antioxidant effects of *Cymbopogon citratus* Stapf (Lemon grass) extract in paracetamol induced hepatotoxicity in rats. *Tropical Journal of Pharmaceutical Research*, 16(1), 101-107.
- Sanjabi, S., Oh, S. A., & Li, M. O. (2017). Regulation of the immune response by TGF- β : from conception to autoimmunity and infection. *Cold Spring Harbor perspectives in biology*, 9(6), a022236.
- Sarges, P., Steinberg, J. M., & Lewis, J. H. (2016). Drug-induced liver injury: highlights from a review of the 2015 literature. *Drug safety*, 39(9), 801-821.
- Schabauer, L., Steflitsch, W., & Buchbauer, G. (2017). Essential oils and compounds against pains in animal studies. *Natural Product Communications*, 12(7): 1137–43.
- Solomon, D. H., Husni, M. E., Libby, P. A., Yeomans, N. D., Lincoff, A. M., Löscher, T. F., & Borer, J. S. (2017). The risk of major NSAID toxicity with celecoxib, ibuprofen, or naproxen: a secondary analysis of the precision trial. *The American Journal of Medicine*, 130(12), 1415-1422.
- Sriuttha, P., Sirichanchuen, B., & Permsuwan, U. (2018). Hepatotoxicity of nonsteroidal anti-inflammatory drugs: A

- systematic review of randomized controlled trials. *International journal of hepatology*, 2018(1), 5253623.
- Stillier, C. O., & Hjendahl, P. (2022). Lessons from 20 years with COX-2 inhibitors: Importance of dose-response considerations and fair play in comparative trials. *Journal of Internal Medicine*, 292(4), 557-574.
- Sussan, T.E., Gajghate, S., Chatterjee, S., Mandke, P., McCormick, S., Sudini, K., Kumar, S., Breyse, P.N., Diette, G.B., Sidhaye, V.K. and Biswal, S. (2015). Nrf2 reduces allergic asthma in mice through enhanced airway epithelial cytoprotective function. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 309(1), L27-L36.
- Taguchi, K., Masui, S., Itoh, T., Miyajima, A., & Yamamoto, M. (2019). Nrf2 activation ameliorates hepatotoxicity induced by a heme synthesis inhibitor. *Toxicological Sciences*, 167(1), 227-238.
- Tan, H. Y., Wang, N., Li, S., Hong, M., Wang, X., & Feng, Y. (2016). The reactive oxygen species in macrophage polarization: reflecting its dual role in progression and treatment of human diseases. *Oxidative medicine and cellular longevity*, 2016(1), 1-16.
- Tanaka, Y., Aleksunes, L. M., Goedken, M. J., Chen, C., Reisman, S. A., Manautou, J. E., & Klaassen, C. D. (2008). Coordinated induction of Nrf2 target genes protects against iron nitrilotriacetate (FeNTA)-induced nephrotoxicity. *Toxicology and applied pharmacology*, 231(3), 364-373.
- Tarkang, A.P., Okalebo, F. A., Ayong, L. S., Agbor, G. A., & Guantai, A. N. (2014). Anti-malarial activity of a polyherbal product (Nefang) during early and established Plasmodium infection in rodent models. *Malaria Journal*, 13, 1-11.
- Tonelli, C., Chio, I. I. C., & Tuveson, D. A. (2018). Transcriptional regulation by Nrf2. *Antioxidants & redox signaling*, 29(17), 1727-1745.
- Umar, M., Mohammed, I., Oko, J., Tafinta, I., Aliko, A., & Jobbi, D. (2016). Phytochemical analysis and antimicrobial effect of lemon grass (*Cymbopogon citratus*) obtained from Zaria, Kaduna State, Nigeria. *Journal of Complementary and Alternative Medical Research*, 1(2), 1-8.
- Urano, A., Matsumaru, D., Ryoke, R., Saito, R., Kadoguchi, S., Saigusa, D., Saito, T., Saido, T.C., Kawashima, R. and Yamamoto, M. (2020). Nrf2 suppresses oxidative stress and inflammation in App knock-in Alzheimer's disease model mice. *Molecular and cellular biology*, 40(6), e00467-19.
- Vega, J., Goecke, H., Méndez, G. P., & Guarda, F. J. (2012). Nephrotic syndrome and acute tubular necrosis due to meloxicam use. *Renal Failure*, 34(10), 1344-1347.
- Wan, E.Y.F., Yu, E.Y.T., Chan, L., Mok, A.H.Y., Wang, Y., Chan, E.W.Y., Wong, I.C.K. and Lam, C.L.K. (2021). Comparative risks of nonsteroidal anti-inflammatory drugs on CKD. *Clinical Journal of the American Society of Nephrology*, 16(6), 898-907.
- Ward, B. S., Naughton, M., Nitsch, D., & Molokhia, M. (2021). The risk of non-steroidal anti-inflammatory drug-induced heart failure in people with chronic kidney disease: a systematic review. *Journal of Public Health*, 1-11.
- Weng, S. E., Hsu, W. T., Hsiao, F. Y., & Lee, C. M. (2024). Non-steroidal anti-inflammatory drugs, renin-angiotensin system blockade or diuretics and risk of acute kidney injury: A case-crossover study. *Archives of Gerontology and Geriatrics*, 123, 105394.
- Weng, S., Hsu, W., Hsiao, F., Lee, C. (2024). 'Non-Steroidal Anti-Inflammatory Drugs, Renin-Angiotensin System Blockade or Diuretics and Risk of Acute Kidney Injury: A Case-Crossover Study'. *Archives of Gerontology and Geriatrics*, 123(202): 105394.
- Wongrakpanich, S., Wongrakpanich, A., Melhado, K., & Rangaswami, J. (2018). A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging and disease*, 9(1), 143-50.
- Wu, J., Pan, X., Fu, H., Zheng, Y., Dai, Y., Yin, Y., Chen, Q., Hao, Q., Bao, D. and Hou, D. (2017). Effect of curcumin on glycerol-induced acute kidney injury in rats. *Scientific reports*, 7(1), 10114.
- Xu, Z., Wei, Y., Gong, J., Cho, H., Park, J. K., Sung, E. R., ... & Duh, E. J. (2014). NRF2 plays a protective role in diabetic retinopathy in mice. *Diabetologia*, 57, 204-213.
- Yagishita, Y., Fukutomi, T., Sugawara, A., Kawamura, H., Takahashi, T., Pi, J., Uruno, A. and Yamamoto, M. (2014). Nrf2 protects pancreatic β -cells from oxidative and nitrosative stress in diabetic model mice. *Diabetes*, 63(2), 605-618.
- Yagishita, Y., Uruno, A., Chartoumpakis, D. V., Kensler, T. W., & Yamamoto, M. (2019). Nrf2 represses the onset of type 1 diabetes in non-obese diabetic mice. *Journal of Endocrinology*, 240(3), 403-416.
- Yagishita, Y., Uruno, A., Fukutomi, T., Saito, R., Saigusa, D., Pi, J., Fukamizu, A., Sugiyama, F., Takahashi, S. and Yamamoto, M. (2017). Nrf2 improves leptin and insulin resistance provoked by hypothalamic oxidative stress. *Cell Reports*, 18(8), 2030-2044.
- Yamamoto, M., Kensler, T. W., & Motohashi, H. (2018). The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiological reviews*, 98(3), 1169-1203.
- Zhao, Z., Liao, G., Zhou, Q., Lv, D., Holthfer, H., & Zou, H. (2016). Sulforaphane attenuates contrast-induced nephropathy in rats via Nrf2/HO-1 pathway. *Oxidative medicine and cellular longevity*, 2016(1), 11-13.
- Zoubek, M. E., Lucena, M. I., Andrade, R. J., & Stephens, C. (2020). Systematic review: ibuprofen-induced liver injury. *Alimentary pharmacology & therapeutics*, 51(6), 603-611.

Full length Research Article

Age-Related Changes in Cognition, Plasma Levels of Brain-Derived Neurotrophic Factor and Selected Indices of Inflammation in Adults at Different Decades of Life

***Rahamon S.K.¹, Yusuff A.O.¹, Ayinde O.O.², Taiwo F.³**

¹Department of Immunology, College of Medicine, University of Ibadan, Nigeria.

²Department of Psychiatry, College of Medicine/University College Hospital, Ibadan, Nigeria.

³Neurology Unit, Department of Medicine, University College Hospital, Ibadan, Nigeria

Summary: Ageing is associated with neurological disorders that are characterized by cognitive impairment. Reports have shown that brain-derived neurotrophic factor (BDNF) is involved in neurogenesis and neuroplasticity. Additionally, chronic inflammation-associated with ageing plays important roles in immunosenescence. However, there is a dearth of information on the interplay between BDNF and inflammation in the context of cognitive decline in the elderly. Thus, this study was designed to determine cognition, plasma levels of BDNF and selected indices of inflammation in human adults at different decades of life. Eighty-eight human adults sub-divided into 4 groups: group I (30 – 39 years), group II (40 – 49 years), group III (50 – 59 years) and group IV (≥ 60 years) were enrolled into this cross-sectional study. Cognitive function was assessed using the Mini-mental state examination (MMSE). Plasma levels of BDNF and nitric oxide (NO) were determined using ELISA and spectrophotometry. White blood cell count and differentials were done using standard methods and neutrophil-lymphocyte ratio (NLR) was calculated appropriately. There was significant reduction in cognitive score and plasma levels of BDNF as decades of life increased. The cognitive scores were significantly higher in groups I ($p = 0.000$), II ($p = 0.000$), and III ($p = 0.000$) compared with group IV. Similarly, the median plasma level of BDNF was higher in group I compared with groups III ($p = 0.007$) and IV ($p = 0.002$). The mean mixed count (monocytes, basophils and eosinophils) was significantly higher in group IV compared with group I ($p = 0.003$), while mean plasma level of NO was higher in group III compared with group I ($p = 0.003$). Age was a significant predictor of cognition ($B = -0.325$, $SE = 0.033$, $p = 0.000$) and BDNF level ($B = -107.660$, $SE = 35.825$, $p = 0.003$). Furthermore, BDNF levels had significant positive correlation with the cognitive score in group I. Conclusively, there was progressive reduction in cognitive score and plasma BDNF level with increasing decades of human life. This may indicate that plasma BDNF level could predict susceptibility to cognitive dysfunction as ageing progresses.

Keywords: Aging, Brain-derived neurotrophic factor, Immunosenescence, Inflammation, Cognition.

*Authors for correspondence: sk.rahamon@ui.edu.ng, adekunlesheu@rocketmail.com; Tel: +234-8067420607

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INTRODUCTION

Ageing is a complex process that is associated with changes in biological, physiological, immunological, psychological, behavioural, and social processes. It is characterized by a progressive and irreversible decline in physical functions resulting from the gradual loss of certain bioprotective and regenerative capabilities (Hernandez-Segura *et al.*, 2018). As ageing progresses, vital organs begin to lose some functions, and all cells change, becoming larger and less able to divide and multiply (Dulken *et al.*, 2019).

Genetic and epigenetic factors play multifaceted roles in the process of ageing and usually result in a reduction in endocrine, immunological, and cognitive functions, among others. It has been reported that gradual loss of cognitive capacity in elderly individuals is linked to changes in the

cortex or hippocampal regions of the brain that are involved in learning and memory (Crook *et al.*, 1987; Kwok, 2010; O'Shea *et al.*, 2016).

Some neuronal loss also occur during normal ageing but usually does not exceed 10% (Morrison and Baxter, 2012). Morphological changes in neurons, especially dendrites and axons, are involved in cognitive decline and behavioural changes (Dickstein *et al.*, 2013). With increasing age, the dendritic trees undergo regression, while the dendritic shafts decrease in number, become shorter and less branched, and have fewer spines (Dickstein *et al.*, 2013).

Several proteins, including brain-derived neurotrophic factor (BDNF), are involved in regulating the growth, survival, and differentiation of neurons. The BDNF is a neuropeptide of the neurotrophin family that can bind to a

specific receptor known as tropomyosin kinase receptor B, activating intracellular signaling pathways that result in the regulation of the growth, survival, and differentiation of neurons (Webster *et al.*, 2006). It is involved in the modulation of synaptic plasticity, including the induction or enhancement of long-term potentiation (Dong *et al.*, 2022), through the promotion of neurogenesis and/or dendritic outgrowth (Pizzorusso *et al.*, 2000). The BDNF is highly expressed in the hippocampus, amygdala, cerebellum and cerebral cortex in both rodents and humans, with the highest levels found in the hippocampus, an area that is involved in learning and memory (Hofer *et al.*, 1990; Miranda *et al.*, 2019). Reports abound indicating that BDNF plays a critical role in maintaining hippocampal volume in adults (Teixeira *et al.*, 2010). It is hypothesized that BDNF influences age-related changes in hippocampal volume through several age-related alterations within the hippocampus. In the central nervous system, BDNF and downstream pro-survival pathways have been demonstrated to protect neurons from damage and enhance neuronal network reorganization after injury (Numakawa *et al.*, 2010). It has also been reported that BDNF treatment can reduce the degree of microglial activation in certain brain injury models, although these responses are considered a consequence of reduced neuronal injury and death elicited by BDNF (Jiang *et al.*, 2011). Additionally, Mizoguchi *et al.* (2020) reported that BDNF hypofunction is associated with age-related memory impairment.

The aging process is accompanied by a state of low-grade non-resolving activation of inflammatory pathways, a phenomenon known as inflammaging (Bowirrat, 2022). This stage is usually associated with an increased number of inflammatory cells in the central nervous system (CNS), thus contributing to a greater degree of neuroinflammation (Stichel and Luebbert, 2007).

Inflammatory pathways can be activated by self-debris and self-molecules that result from unhealthy or dead cells produced at a relatively high rate in aged tissues, the accumulation of senescent cells and their associated proinflammatory secretome (Garthwaite *et al.*, 1988; Sama and Norris, 2013). Several age-related synaptic alterations in Ca²⁺ homeostasis may be responsible for the elevation of intracellular Ca²⁺ and neuroinflammation, as indicated by the production of proinflammatory cytokines, including interleukin-1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) (Lourenço *et al.*, 2017). Cellular markers of inflammation, such as the neutrophil-lymphocyte ratio (NLR), positively correlate with age. Older people have been observed to have elevated NLRs compared with young adults (Li *et al.*, 2015).

Nitric oxide (NO) plays important roles in several neurobiological processes, ranging from the regulation of endothelium-dependent vasodilation to neurotransmission, and participates in host defence mechanisms (Boje, 2004). Because it has been described to be both neuroprotective and neurotoxic, it has been referred to as a Janus-faced molecule (Toledo and Augusto, 2012). It is a crucial component of the signal transduction pathways used for memory formation, sensory processing, and the regulation of cerebral blood flow (Bernard-Gauthier *et al.*, 2013). NO signalling in the ageing brain revealed that a small decrease in neuronal-derived NO in the aged hippocampus was accompanied by a more robust decrease in the

cerebrovascular response produced upon stimulation of neuronal activity. It has been shown that there is decreased nitric oxide synthase (NOS) expression with age, both as constitutive and inducible isoforms (Lourenço *et al.*, 2017). An increase in mediators of inflammation reduces BDNF expression, and BDNF may play an important negative regulatory role in inflammation within the brain (Schramm *et al.*, 2002). Currently, there is a dearth of information on age-related changes in BDNF levels and inflammation in Nigerian adults. Thus, this study was designed to determine the relationship between cognition, plasma BDNF levels and selected indices of inflammation in adults of different decades of life to provide information that could be of therapeutic importance to age-related neurological status in health and disease conditions.

MATERIALS AND METHODS

Human ethics and consent to participate: This study was carried out in accordance with the Declaration of Helsinki and approved by the University of Ibadan/University College Hospital Joint Ethics Committee (UI/EC/21/0698). Additionally, written informed consent was obtained from each study participant.

Study participants and location: A total of 88 apparently healthy adults (39 males, 49 females) were enrolled in this cross-sectional study. The participants consisted of 20, 22, 22 and 24 adults within the age ranges of 30–39 years (Group I), 40–49 years (Group II), 50–59 years (Group III), and 60 years and above (Group IV), respectively. The participants were enrolled using a convenient sampling method from the metropolis of Ibadan city, Nigeria.

Assessment of neurocognition: Neurocognition was assessed using the Mini-Mental State Examination (MMSE), a neuropsychological screening test used to assess the speed of information processing, executive function, learning, memory, motor function, and verbal function. The test yields a single composite score that reflects disease severity. Scores <10, 10–20, 21–24 and ≥ 25 indicate severe impairment, moderate impairment, mild impairment and no impairment, respectively (Folstein *et al.*, 1975).

Inclusion and exclusion criteria: Apparently healthy adults within the age range of 30 years and above with no history of stroke, posttraumatic stress disorder, learning disability, mental illness, substance abuse and/or dependence, metabolic and cardiovascular diseases or autoimmunity were enrolled into this study. Patients with HIV or CNS opportunistic infections such as toxoplasmosis and meningitis were excluded from the study. Additionally, patients with a history of previous or present smoking and alcohol consumption and patients who recently used anti-inflammatory drugs and steroids were excluded from the study.

Blood sample collection: Venous blood (5 ml) was collected from the antecubital fossa of each participant, using needle and syringe, after at least 10 minutes of rest and dispensed into heparin-containing sample bottles. Twenty-five microliters (25 μ L) of whole blood was taken for the nitroblue tetrazolium (NBT) assay. The remaining

blood sample was centrifuged at 3000 rpm for 15 minutes, and the obtained plasma samples were stored at -20°C until analysis.

Laboratory analysis: The plasma BDNF level was determined using a Sandwich ELISA following the manufacturer's instructions (ElabScience Biotechnology, Inc., USA). The plasma level of NO was determined using the Griess reagent as described by Green *et al.* (1982), while the neutrophil phagocytic activity was determined using the modified semiquantitative NBT procedure as described by Edem and Arinola (2015). Complete blood analysis was performed using a 3-parameter Hematology Autoanalyzer (Mindray BC-5390, Shenzhen Mindray Bio-Medical Electronics Co., China). Thereafter, the neutrophil-to-lymphocyte ratio (NLR) was calculated as the ratio of the neutrophil count (%) to the lymphocyte count (%) (Li *et al.*, 2015).

Data analysis: The Statistical Package for Social Sciences (SPSS), version 23.0, was used for data analysis. The data were assessed for a Gaussian distribution; thereafter, an appropriate statistical tool was applied. The Gaussian distribution of the data was assessed using a histogram with a normal curve. Mean differences between the groups (for variables with Gaussian distributions) were determined using ANOVA followed by LSD post hoc tests. However, the Kruskal–Wallis and Mann–Whitney U tests were used to determine differences in median values between the groups (for variables with a non-Gaussian distribution). Correlations between the variables were determined using the Spearman rho correlation. Linear regression analysis was used to predict the relationship between the age (as an independent variable) and cognitive score (dependent variable) as well as age and plasma BDNF level (dependent variable). P values less than 0.05 were considered to indicate statistical significance. Results are presented as mean $\pm$ standard deviation or median (interquartile range), as appropriate.

RESULTS

The age of the study participants ranged between 31 and 82 years, with a mean of 52.28 ± 13.63 years. As shown in Figures 1 and 2, there was a significant reduction in the neurocognitive score (p-value = 0.000) and plasma BDNF level (p-value = 0.009) as the age increased. The neurocognitive scores were significantly higher in Group I (29.35 ± 0.67), Group II (28.00 ± 1.72), and Group III (26.68 ± 2.32) than in Group IV (19.17 ± 8.48). Similarly, the median plasma BDNF level was significantly higher in Group I [8561.65 (5895-10310.90)] than in Groups III [5082.72 (3154.31-5360.06), p-value = 0.007] and IV [4630.59 (1758.43-4630.59), p-value = 0.002].

In Table 1, there were significant differences in the mean plasma levels of NO and mixed count among the four groups. The mean mixed count (monocytes, basophils and eosinophils) was significantly higher in Group IV than in Group I (p-value = 0.003), while the mean plasma NO level was significantly higher in Group III than in Group I (p-value = 0.003).

In Table 2, correlations between the neurocognitive score and other parameters are presented. The median plasma level of BDNF level had significant positive correlation with the neurocognitive score in Group I. None of the other parameters were significantly correlated with the neurocognitive score.

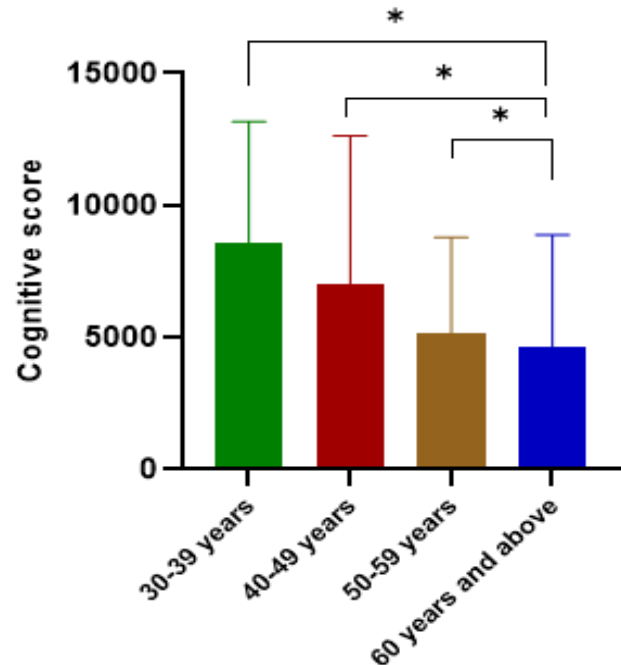


Figure 1: Cognitive score in adults at different decades of human life
*Significant at $p < 0.05$

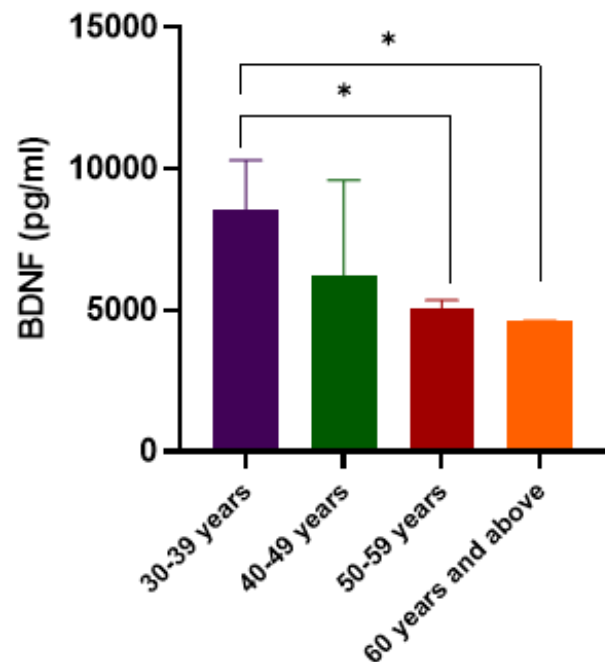


Figure 2: Plasma BDNF levels in adults at different decades of human life

Table 1:

White blood cell count, neutrophil-to-lymphocyte ratio and plasma nitric oxide level in adults in different decades

Parameters	Group I (n = 20)	Group II (n = 22)	Group III (n = 22)	Group IV (n = 24)	p-value
WBC count (cmm)	6267 ± 2414	7547 ± 3501	819 5± 3465	6727 ± 1622	0.200
Neutrophil count (%)	44.42 ± 13.70	53.45 ± 13.87	53.31 ± 12.21	46.90 ± 11.85	0.088
Lymphocytes count (%)	40.26 ± 11.07	39.07 ± 14.29	37.28 ± 18.24	40.39 ± 9.38	0.898
Mixed count (%)	5.75 (3.63-9.78) ^a	8.35 (2.73 - 10.08)	8.60 (5.30 - 11.20)	12.10 (9.40 - 15.40)	0.021*
NLR	1.11 (0.63 - 1.92) ^a	1.54 (0.97 - 1.86)	1.35 (0.81 - 2.19)	1.02 (0.81 - 1.60)	0.231
NO (μM)	0.85 (0.83-0.89) ^b	0.92 (0.84-1.09)	0.93 (0.88-0.98)	0.87 (0.85 - 0.92)	0.011*

*Significant at $P < 0.05$, WBC = white blood cell, cmm = cubic millimeter, Mixed = other leucocytes, NLR = neutrophil-to-lymphocyte ratio, NO = nitric oxide. ^aCompared with Group IV, ^bCompared with Group III, Group I = 30-39 years, Group II = 40-49 years, Group III = 50-59 years, Group IV = 60 years and above

Table 2:

Correlations between the neurocognitive score, neutrophil-to-lymphocyte ratio and plasma BDNF level concentration

Parameters	Group I (n = 20)		Group II (n = 22)		Group III (n = 22)		Group IV (n = 24)	
	MMSE							
	r	p	r	p	r	p	r	p
BDNF (pg/ml)	0.604	0.013*	0.402	0.098	0.233	0.368	0.315	0.294
NO (μM)	-0.237	0.315	0.166	0.460	-0.399	0.066	0.033	0.878
NLR	0.318	0.198	-0.094	0.701	-0.121	0.613	-0.226	0.417

*Significant at $P < 0.05$; MMSE = Mini-Mental State Examination; NLR = neutrophil-to-lymphocyte ratio; BDNF = brain-derived neurotrophic factor; NO = nitric oxide, r = correlation coefficient; Group I = 30-39 years, Group II = 40-49 years, Group III = 50-59 years, Group IV = 60 years and above.

Table 3:

Linear regression analysis of age and cognition in the study participants

Predictor	Unstandardized Coefficients		Standardized Coefficient	t-value	p-value
	B	Standard error	Beta		
Constant	42.545	1.808	—	23.531	0.000*
Age (years)	-0.325	0.033	-0.723	-9.700	0.000*
R^2					0.522
F					94.093
P					0.000*
Regression equation: Cognitive score = 42.545 + [-0.325 (Age)]					

*Significant at $P < 0.05$, Dependent variable- Cognitive score, Predictor variable- Age

Table 4:

Linear regression analysis of age and BDNF level in the study participants

Predictor	Unstandardized Coefficients		Standardized Coefficient	t-value	p-value
	B	Standard error	Beta		
Constant	11875.107	1935.022	—	6.137	0.000*
Age (years)	-107.660	35.825	-0.308	-3.005	0.003*
R^2					0.095
F					9.031
P					0.003*
Regression equation: Plasma BDNF level = 11875.107 + [-107.66 (Age)]					

*Significant at $P < 0.05$, Dependent variable- BDNF (pg/ml), Predictor variable- Age, BDNF = Brain-derived neurotrophic factor

As presented in Tables 3 and 4, age was a significant predictor of cognition ($B = -0.325$, $SE = 0.033$, $p = 0.000$) and BDNF level ($B = -107.660$, $SE = 35.825$, $p = 0.003$). This indicates that, for every year increase in the age of the study participants, cognition decreased by 0.325, while BDNF level decreased by 107.66 pg/ml. The proportion of variance in cognitive score that could be explained by age was 52.2%, while the proportion of variance in plasma BDNF levels that could be explained by age was 9.5%.

Age-Related Changes in Cognition, BDNF, and Inflammation

DISCUSSION

It is well established that cognitive function is affected by ageing, as approximately 40% of individuals aged 65 years and older suffer from some form of memory loss (Aigbogun *et al.*, 2017; Brito *et al.*, 2023). Although several factors have been identified, the mechanisms through which the identified factors result in cognitive dysfunction are poorly understood.

This study observed a significant decrease in cognitive score as the duration of life increased. The regression analysis further supported this, showing that age was negatively related to cognition. These observations corroborate the findings of Park *et al.* (2002), who showed that there are gradual age-related declines in the cognitive mechanisms of speed, working memory, and long-term memory beginning in young adulthood. The observations from this study could be due to a decrease in neuronal activation and alterations in several regions of the brain as ageing progresses. Brain activity in regions such as the cortex, hippocampus and cerebellum is altered during ageing (Calautti *et al.*, 2001; Brito *et al.*, 2020). This results in a decrease in the speed of processing, working memory, inhibitory function, and long-term memory (Park and Reuter-Lorenz, 2009; Randhawa and Varghese, 2024).

The BDNF is involved in plasticity, neuronal survival, formation of new synapses, dendritic branching, and modulation of excitatory and inhibitory neurotransmitter profiles (Edelmann *et al.*, 2014). A decrease in its level has been associated with age-related memory impairment (Panja *et al.*, 2014). In this study, there was a progressive decrease in the plasma BDNF concentration as the subjects aged. The plasma BDNF levels were significantly lower in participants aged 50 years and older than in those who were younger. Additionally, a negative relationship was observed between age and BDNF level. These observations are in line with the findings of Mizoguchi *et al.* (2020), who showed that low serum BDNF levels are associated with age-related memory impairment in elderly individuals. Although a reduction in BDNF expression has been associated with chronic inflammation, which is a common feature in elderly individuals (2011), the findings of this study might not be inflammation related, as no significant difference in the NLR was observed when all the groups were compared. The observed reduced plasma BDNF could, therefore, be due to decreased physical activity, which is common in elderly people. BDNF levels are activity-dependent (Gorgoulis *et al.*, 2019). Exercise is an inducer of neuronal plasticity, neurogenesis and survival (Huang *et al.*, 2014). Furthermore, the observed low level of BDNF in aged individuals could be diet-dependent, as poor appetite is a common problem in older people (Pilgrim *et al.*, 2015). A study by Gorgoulis *et al.* (2019) showed that diet can provoke changes in BDNF levels under physiological conditions. Low BDNF levels have been reported in adults consuming high sugar and fat-containing diets (Mizoguchi *et al.* (2020), while diets rich in omega-3 fatty acids have been shown to induce an increase in BDNF levels and a reduction in cognitive impairment (Wu *et al.*, 2004; Mizoguchi *et al.*, 2020).

The observed significant positive correlation between BDNF level and cognitive score indicated that an increase in BDNF level may be associated with improved cognition. This observation is not surprising, as the interplay between BDNF and cognition is well established. BDNF could therefore be considered a potential therapy for brain pathologies because it can directly or indirectly modulate changes within the brain (Nagahara and Tuszynski, 2011; Gorgoulis *et al.*, 2019). Nagahara and Tuszynski (2011) reported that BDNF has a potential role in the pathogenesis and treatment of both neurological and psychiatric disorders.

Chronic inflammation is a common feature of ageing. Cellular markers of inflammation, such as the neutrophil-to-lymphocyte ratio (NLR), have been shown to correlate positively with age. In this study, no significant difference in the NLR was observed between the groups. This observation contradicts the findings of Li *et al.* (Li *et al.*, 2015), who showed that older people have a higher NLR than young adults. These differences could be due to variations in the selection of study participants, as participants in this study were grouped according to decade of life, and age-associated chronic inflammation might not be linear.

Nitric oxide is a mediator and regulator of inflammatory responses (Sharma *et al.*, 2007). The function of NO in the hypothalamus has largely been implicated in learning processes and memory formation. In this study, the mean plasma NO concentration was significantly higher in participants aged 40–49 years (Group II) and 50–59 years (Group III) than in participants aged 30–39 years (Group I). This contradicts the findings of Van der Loo *et al.* (2000), who showed that the bioavailability or generation of NOS-derived NO decreases with age. However, Siervo *et al.* (2018) reported that age-related changes in serum NO levels peaked between 50 and 59 years in both sexes and declined thereafter. This finding is similar to what was observed in this study, in which the NO concentration decreased in participants aged 60 years and older. Although this observation could not be fully explained at present, it might indicate that the serum NO concentration reflects the well-established immunosenescence pattern.

The small sample size was a limitation in this study. Additionally, the use of only the MMSE to assess cognitive function was a limitation. The MMSE has been reported to have low sensitivity for detecting mild cognitive disorders, and it is largely a screening tool.

It could be concluded from this study that there is a progressive reduction in the plasma BDNF concentration and cognitive score with increasing decades of life. This may indicate that the plasma BDNF concentration could predict susceptibility to cognitive impairment as aging progresses and could be further explored as a potential neuroprotective and functional restorative factor in patients with cognitive dysfunction. However, large population studies are suggested to confirm the findings of this study. Similarly, a randomized blinded clinical trial is suggested to evaluate the therapeutic effect of BDNF administration on improving cognition and preventing dementia in elderly people.

REFERENCES

- Aigbogun, M.S., Stellhorn, R., Krasa, H., Kostic, D. (2017). Severity of memory impairment in the elderly: Association with health care resource use and functional limitations in the united states. *Alzheimers Dement (Amst)*. 8:51-59.
- Bernard-Gauthier, V., Boudjemeline, M., Rosa-Neto, P., Thiel, A., Schirmacher, R. (2013). Towards tropomyosin-related kinase b (trkb) receptor ligands for brain imaging with pet: Radiosynthesis and evaluation of 2-(4-[(18)f]fluorophenyl)-7,8-dihydroxy-4h-chromen-4-one and 2-(4-[(n-methyl-(11)c]-dimethylamino)phenyl)-7,8-dihydroxy-4h-chromen-4-one. *Bioorg Med Chem*. 2124:7816-7829.

- Boje, K.M. (2004). Nitric oxide neurotoxicity in neurodegenerative diseases. *Front Biosci.* 9:763-776.
- Bowirrat, A. (2022). Immunosenescence and aging: Neuroinflammation is a prominent feature of alzheimer's disease and is a likely contributor to neurodegenerative disease pathogenesis. *J Pers Med.* 1211.
- Brito, D., Esteves, F., Rajado, A., Silva, N., Araújo, I., Bragança, J., et al. (2023). Assessing cognitive decline in the aging brain: Lessons from rodent and human studies. *NPJ Aging.* 91:23.
- Brito, D.V.C., Gulmez Karaca, K., Kupke, J., Mudlaff, F., Zeuch, B., Gomes, R., et al. (2020). Modeling human age-associated increase in gadd45 γ expression leads to spatial recognition memory impairments in young adult mice. *Neurobiol Aging.* 94:281-286.
- Calautti, C., Serrati, C., Baron, J.C. (2001). Effects of age on brain activation during auditory-cued thumb-to-index opposition: A positron emission tomography study. *Stroke.* 321:139-146.
- Crook, T., Bahar, H., Sudilovsky, A. (1987). Age-associated memory impairment: Diagnostic criteria and treatment strategies. *Int J Neurol.* 21-22:73-82.
- Dickstein, D.L., Weaver, C.M., Luebke, J.I., Hof, P.R. (2013). Dendritic spine changes associated with normal aging. *Neuroscience.* 251:21-32.
- Dong, T.N., Kramár, E.A., Beardwood, J.H., Al-Shammari, A., Wood, M.A., Keiser, A.A. (2022). Temporal endurance of exercise-induced benefits on hippocampus-dependent memory and synaptic plasticity in female mice. *Neurobiol Learn Mem.* 194:107658.
- Dulken, B.W., Buckley, M.T., Navarro Negredo, P., Saligrama, N., Cayrol, R., Leeman, D.S., et al. (2019). Single-cell analysis reveals t cell infiltration in old neurogenic niches. *Nature.* 5717764:205-210.
- Edelmann, E., Lessmann, V., Brigadski, T. (2014). Pre- and postsynaptic twists in bdnf secretion and action in synaptic plasticity. *Neuropharmacology.* 76 Pt C:610-627.
- Edem, V.F., Arinola, G.O. (2015). Innate cellular immunity in newly diagnosed pulmonary tuberculosis patients and during chemotherapy. *Ann Glob Health.* 815:669-674.
- Folstein, M.F., Folstein, S.E., McHugh, P.R. (1975). "Minimal state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 123:189-198.
- Garthwaite, J., Charles, S.L., Chess-Williams, R. (1988). Endothelium-derived relaxing factor release on activation of nmda receptors suggests role as intercellular messenger in the brain. *Nature.* 3366197:385-388.
- Gorgoulis, V., Adams, P.D., Alimonti, A., Bennett, D.C., Bischof, O., Bishop, C., et al. (2019). Cellular senescence: Defining a path forward. *Cell.* 1794:813-827.
- Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite, and [15n]nitrate in biological fluids. *Anal Biochem.* 1261:131-138.
- Hernandez-Segura, A., Nehme, J., Demaria, M. (2018). Hallmarks of cellular senescence. *Trends Cell Biol.* 286:436-453.
- Hofer, M., Pagliusi, S.R., Hohn, A., Leibrock, J., Barde, Y.A. (1990). Regional distribution of brain-derived neurotrophic factor mrna in the adult mouse brain. *Embo j.* 98:2459-2464.
- Huang, T., Larsen, K.T., Ried-Larsen, M., Møller, N.C., Andersen, L.B. (2014). The effects of physical activity and exercise on brain-derived neurotrophic factor in healthy humans: A review. *Scand J Med Sci Sports.* 241:1-10.
- Jiang, Y., Wei, N., Lu, T., Zhu, J., Xu, G., Liu, X. (2011). Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. *Neuroscience.* 172:398-405.
- Kwok, J.B. (2010). Role of epigenetics in alzheimer's and parkinson's disease. *Epigenomics.* 25:671-682.
- Li, J., Chen, Q., Luo, X., Hong, J., Pan, K., Lin, X., et al. (2015). Neutrophil-to-lymphocyte ratio positively correlates to age in healthy population. *J Clin Lab Anal.* 296:437-443.
- Lourenço, C.F., Ledo, A., Barbosa, R.M., Laranjinha, J. (2017). Neurovascular-neuroenergetic coupling axis in the brain: Master regulation by nitric oxide and consequences in aging and neurodegeneration. *Free Radic Biol Med.* 108:668-682.
- Miranda, M., Morici, J.F., Zanoni, M.B., Bekinshtein, P. (2019). Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Front Cell Neurosci.* 13:363.
- Mizoguchi, Y., Yao, H., Imamura, Y., Hashimoto, M., Monji, A. (2020). Lower brain-derived neurotrophic factor levels are associated with age-related memory impairment in community-dwelling older adults: The sefuri study. *Sci Rep.* 101:16442.
- Morrison, J.H., Baxter, M.G. (2012). The ageing cortical synapse: Hallmarks and implications for cognitive decline. *Nat Rev Neurosci.* 134:240-250.
- Nagahara, A.H., Tuszynski, M.H. (2011). Potential therapeutic uses of bdnf in neurological and psychiatric disorders. *Nat Rev Drug Discov.* 103:209-219.
- Numakawa, T., Suzuki, S., Kumamaru, E., Adachi, N., Richards, M., Kunugi, H. (2010). Bdnf function and intracellular signaling in neurons. *Histol Histopathol.* 252:237-258.
- O'Shea, A., Cohen, R.A., Porges, E.C., Nissim, N.R., Woods, A.J. (2016). Cognitive aging and the hippocampus in older adults. *Front Aging Neurosci.* 8:298.
- Panja, D., Kenney, J.W., D'Andrea, L., Zalfa, F., Vedeler, A., Wibrand, K., et al. (2014). Two-stage translational control of dentate gyrus ltp consolidation is mediated by sustained bdnf-trkb signaling to mnk. *Cell Rep.* 94:1430-1445.
- Park, D.C., Lautenschlager, G., Hedden, T., Davidson, N.S., Smith, A.D., Smith, P.K. (2002). Models of visuospatial and verbal memory across the adult life span. *Psychol Aging.* 172:299-320.
- Park, D.C., Reuter-Lorenz, P. (2009). The adaptive brain: Aging and neurocognitive scaffolding. *Annu Rev Psychol.* 60:173-196.
- Pilgrim, A.L., Robinson, S.M., Sayer, A.A., Roberts, H.C. (2015). An overview of appetite decline in older people. *Nurs Older People.* 275:29-35.
- Pizzorusso, T., Ratto, G.M., Putignano, E., Maffei, L. (2000). Brain-derived neurotrophic factor causes camp response element-binding protein phosphorylation in absence of calcium increases in slices and cultured neurons from rat visual cortex. *J Neurosci.* 208:2809-2816.
- Randhawa, S.S., Varghese, D. (2024). Geriatric evaluation and treatment of age-related cognitive decline Statpearls. Treasure Island (FL) ineligible companies. Disclosure: Dona Varghese declares no relevant financial relationships with ineligible companies.: *StatPearls Publishing LLC.*
- Sama, D.M., Norris, C.M. (2013). Calcium dysregulation and neuroinflammation: Discrete and integrated mechanisms for age-related synaptic dysfunction. *Ageing Res Rev.* 124:982-995.
- Schramm, U., Berger, G., Müller, R., Kratzsch, T., Peters, J., Frölich, L. (2002). Psychometric properties of clock drawing test and mmse or short performance test (skt) in dementia screening in a memory clinic population. *Int J Geriatr Psychiatry.* 173:254-260.

- Sharma, J.N., Al-Omran, A., Parvathy, S.S. (2007). Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*. 156:252-259.
- Siervo, M., Scialò, F., Shannon, O.M., Stephan, B.C.M., Ashor, A.W. (2018). Does dietary nitrate say no to cardiovascular ageing? Current evidence and implications for research. *Proc Nutr Soc*. 772:112-123.
- Stichel, C.C., Luebbert, H. (2007). Inflammatory processes in the aging mouse brain: Participation of dendritic cells and t-cells. *Neurobiol Aging*. 2810:1507-1521.
- Teixeira, A.L., Barbosa, I.G., Diniz, B.S., Kummer, A. (2010). Circulating levels of brain-derived neurotrophic factor: Correlation with mood, cognition and motor function. *Biomark Med*. 46:871-887.
- Toledo, J.C., Jr., Augusto, O. (2012). Connecting the chemical and biological properties of nitric oxide. *Chem Res Toxicol*. 255:975-989.
- van der Loo, B., Labugger, R., Skepper, J.N., Bachschmid, M., Kilo, J., Powell, J.M., et al. (2000). Enhanced peroxynitrite formation is associated with vascular aging. *J Exp Med*. 19212:1731-1744.
- Webster, M.J., Herman, M.M., Kleinman, J.E., Shannon Weickert, C. (2006). Bdnf and trkb mrna expression in the hippocampus and temporal cortex during the human lifespan. *Gene Expr Patterns*. 68:941-951.
- Wu, A., Ying, Z., Gomez-Pinilla, F. (2004). Dietary omega-3 fatty acids normalize bdnf levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. *J Neurotrauma*. 2110:1457-1467.

Full length Research Article

Carvedilol and Clomiphene Combination Therapy Alleviates Inflammation and Redox Imbalance in Experimental PCOS: Role of Nrf2/HMOX-1 and Nfkb Signaling

Ajibare A.J.¹, Akintoye O.O.², Oriyomi A.I.², Orugun S.T.³, *Odetayo A.F.⁴,
Busari A.M.⁴, Olayaki L.A.⁵

¹Department of Physiology, College of Medicine, Lead City University, Ibadan, Nigeria

²Department of Physiology, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria

³Department of Internal Medicine, Ladoke Akintola University Teaching Hospital, Ogbomosho, Nigeria.

⁴Department of Physiology, Federal University of Health Sciences, Ila Orangun, Nigeria.

⁵Department of Physiology, University of Ilorin, Ilorin, Nigeria

Summary: Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenemia, irregular menstrual cycle, and small cysts on the ovaries. This condition can be morphological or biochemical (elevated testosterone). Elevated testosterone (hyperandrogenemia) is the hallmark of PCOS, which can inhibit follicular development, anovulation, or cause irregular menstrual changes. Unfortunately, there is no cure for PCOS, and available treatment options are restricted to mitigating its symptoms. This study was, however, designed to investigate the synergistic effect of clomiphene (CLO) and carvedilol (CAL) on PCOS-induced female infertility. Thirty female Wistar rats were randomized into 5 groups (n= 6/group) control, PCOS, PCOS+ CLO, PCOS+CAL, and PCOS+ CLO+CAL. The administration was once daily via the oral route and lasted for 15 days. Clomiphene and carvedilol synergistically ameliorated PCOS-induced elevated serum gonadotropin-releasing hormone, luteinizing hormone (LH), testosterone and prolactin, and decreased follicle stimulating hormone (FSH), estrogen and progesterone. This was accompanied by the downregulation of PCOS-induced overexpression of ovarian LH, androgen, and FSH receptors. It was also accompanied by a decrease in inflammatory markers such as ovarian interleukin 1 beta and Nuclear factor kappa B (NF-κB) and apoptosis markers such as ovarian caspase 3 and an increase in ovarian Nuclear factor erythroid-2-related factor 2 (Nrf2), Heme Oxygenase 1 (HO 1 or HMO-1), catalase and glutathione reductase. This study shows that carvedilol and clomiphene combination therapy alleviates inflammation and redox imbalance in experimental PCOS.

Keywords: Hormonal imbalance; Apoptosis; Folliculogenesis; Infertility, Polycystic Ovarian Syndrome

*Authors for correspondence: adeyemidetayo@gmail.com, Tel: +234-7032766035

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a multifaceted and varied disorder that affects women in their reproductive years, with a global prevalence of 5-10% (Joham *et al.*, 2022). It is a significant contributor to infertility and is characterized by the appearance of numerous small cysts on the ovaries, menstrual irregularities, and hyperandrogenism (Chaudhuri, 2023). The precise pathophysiology of PCOS is still unclear, but it is thought to result from a complex interplay of genetic, environmental, and lifestyle factors (Dabadghao, 2019). Unfortunately, there is no cure for PCOS, and available treatment options are restricted to mitigating its symptoms (Al Khalifa, 2021). Based on current knowledge, the mechanistic basis of PCOS, though unclear, is closely related to oxidative stress, inflammation,

and endocrine dysfunction (Yeon *et al.*, 2010; Akintoye *et al.*, 2023).

Nuclear factor erythroid-2-related factor 2 (Nrf2)/Heme Oxygenase 1 (HO 1) signaling protects the body system from oxidative stress. Nrf2 is considered a major defense mechanism from oxidative stress by stimulating the removal of reactive oxygen species (ROS) via the activation of genes encoding the phase II detoxifying and antioxidant enzymes such as HMOX-1 (Xiong *et al.*, 2015). Also, Nuclear factor kappa B (NF-κB) promotes inflammation through the transcriptional induction of pro-inflammatory cytokines and chemokines (Sun *et al.*, 2013). These pro-inflammatory cytokines can induce inflammation directly or stimulate the differentiation of inflammatory T cells (Liu *et al.*, 2017).

Pharmacological interventions, such as oral contraceptive pills and anti-androgen administration, have

been utilized to manage the symptoms of PCOS (Chouhan, 2022). Fertility treatments such as ovulation induction with clomiphene citrate (CLO) have also been employed. While these treatments can be efficacious in addressing specific symptoms, PCOS remains an incurable condition (Alesi *et al.*, 2022). As such, there is an urgent need for ongoing research to identify more effective treatments that target the underlying pathophysiological mechanisms of this disorder (Xu *et al.*, 2022). It was predicted that combining drugs with different mechanisms of action could be more advantageous in PCOS management rather than a monotherapy treatment. Hence, this study was designed to explore the synergistic effect of carvedilol (CAR) and CLO in PCOS animals.

CLO is a selective estrogen receptor modulator that promotes ovulation and ameliorates infertility in women with PCOS (Cunha and Póvoa, 2021). Although CLO is the first line pharmacological therapy for ovulation induction in women with PCOS, it still possess some side effects such as multiple pregnancies. Also, CLO may not be effective for treating oxidative stress (Wahab, 2019) which is part of PCOS pathogenesis. CAL is a beta-blocker with ability to prevent hyperandrogenism, a hallmark of PCOS (Aikoye *et al.*, 2019). It has also been shown to possess properties as a modulator of redox and inflammatory signaling pathways and an enhancer of insulin sensitivity; these factors are all key drivers in the pathophysiology of PCOS (Bakr *et al.*, 2022). Despite the potential complementarity of their mechanisms of action, the combination of carvedilol and clomiphene has yet to be extensively investigated as a possible treatment option for PCOS. Therefore, co-administration of these medications may provide a multifaceted approach to addressing the complex nature of PCOS. Hence, this study investigated the synergistic effect of carvedilol and clomiphene in ameliorating PCOS-induced endocrine dysfunction in an experimental rat model. Furthermore, the study explored the role of Nrf2/HMOX-1 and NF- κ B signaling as the possible mechanism of action.

MATERIALS AND METHODS

Drugs and Chemicals: The drugs utilized in this study were acquired from reputable sources. Letrozole, manufactured by Novartis Pharmaceuticals in India, was utilized for the study. Carvedilol was procured from Ciron Drugs and Pharmaceuticals, also located in India. Clomiphene citrate tablets were obtained from Doppel Farmaceutici Sri Italy. Furthermore, ELISA kits, such as Gonadotropin-releasing hormone (GnRH), Follicle Stimulating Hormone (FSH), Luteinizing hormone (LH), Testosterone, Estrogen, Progesterone, Prolactin, and Catalase, were purchased from Bio-Inteco in the United Kingdom

Animals: For this study, thirty virgin female Wistar rats weighing 190-210g were kept at $25 \pm 2^\circ\text{C}$ and maintained on a 12-hour light-dark cycle. Rat chow and water were made available ad libitum. During the study, strict adherence was held to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals to ensure the welfare and ethical treatment of the animals.

Experimental method: At the onset of the study, the animals were divided into five groups consisting of six

animals each. Group 1 (control) received a daily oral dose of 1 mg/kg of distilled water throughout the study. The remaining animals were induced with polycystic ovary syndrome (PCOS) by administering a daily oral dose of 1 mg/kg of letrozole dissolved in distilled water for 21 days (Marouf *et al.*, 2022; Akintoye *et al.*, 2023). After PCOS induction, the animals were randomly divided into four treatment groups as follows: Group 2 (PCOS untreated) received oral doses of 1 mg/kg of distilled water, Group 3 received clomiphene citrate in distilled water at 1 mg/kg orally (PCOS treated with CLO), Group 4 received carvedilol 10 mg/kg in distilled water by oral gavage (PCOS treated with CAL), and Group 5 received 1 mg/kg clomiphene citrate and 10 mg/kg carvedilol by oral gavage (PCOS treated with CLO and CAL). All treatment groups received their respective treatments for 15 days, and overnight fasted animals were euthanized via intraperitoneal administration of 4 mg/kg xylazine and 40 mg/kg of ketamine. The dosages of CLO and CAL are similar with previously reported doses (Akintoye *et al.*, 2023). The ovaries were also harvested, trimmed off of surrounding tissues, and kept in Bouin solution for histological processing.

Estrous phase determination: PCOS induction was confirmed using estrus cycle. The estrous phase of estrus cycle was determined through vaginal smears collected daily for 21 days between 7 am and 9 am to minimize variations due to circadian rhythms (Akintoye *et al.*, 2023). To collect the smears, approximately 40 μl of normal saline was injected into the vagina of the rats using a micropipette and then aspirated in. Afterward, about 15 μl of the fluid aspirated from the vagina was placed onto a clean, air-dried slide and analyzed under a light microscope with 40x objective lenses. The estrous phase was determined based on the proportion of three types of cells: leukocytes (little round ones), epithelial cells (round and nucleated), and cornified cells (irregular anucleated ones).

Biochemical analysis: Blood samples were collected from the experimental rats through cardiac puncture 24 h after the last treatment dose. The blood samples were stored in plain bottles and separated by centrifugation at 3000 rpm/min for 15 minutes and was used for hormonal assays. The left ovaries were also harvested, homogenized in cold phosphate buffer solution and stored for biochemical analysis (Interleukin 1 B (IL-1B) and catalase).

Ovarian tissue gene expression: Right ovaries were harvested from the female rats and dissected into two longitudinal halves. The manufacturer's instructions were followed to isolate total RNA using TRIzol Reagent (ThermoFisher Scientific). DNase I treatment (ThermoFisher Scientific) was applied to remove extracted RNA. DNA-free RNA was instantly transcribed into cDNA using the ProtoScript® First Strand cDNA Synthesis Kit (NEB). Polymerase Chain Reaction (PCR) amplification was performed using OneTaq® 2X Master Mix (NEB) with the following forward and reverse primer sets used: Nuclear factor-erythroid factor 2-related factor 2, HO-1, Glutathione reductase (GSR), Follicle-stimulating hormone receptor (FSHR), Luteinizing hormone receptor (LHR), Androgen receptor (AR), NF- κ B, and Caspase 3.

Forward and Reverse Primers**NRF2:**

Forward primer	GTCAGCTACTCCCAGGTTGC
Reverse primer	CAGGGCAAGCGACTGAAATG

HO-1:

Forward primer	CGACAGCATGTCCCAGGATT
Reverse primer	AGGAGGCCATCACCAGCTTA

GSR

Forward primer	CGGAAACTCGCCCATAGACT
Reverse primer	TGGACGGCTTCATCTTCAGT

FSHr

Forward primer	CATTCTGGGACACGGGATCT
Reverse primer	GGTGAGCACAAACCTCAGTTC

LHr

Forward primer	TCACAGCTGCAGTCCCGAG
Reverse primer	GGGAGATAGGTGAGAGATAGTCTGG

AR

Forward primer	AATGTACAGCCAGTGCGTGA
Reverse primer	GCCCATCCACTGGAATAATGC

NFKB

Forward primer	TTCAACATGGCAGACGACGA
Reverse primer	AGGTATGGGCCATCTGTTGAC

Casp 3

Forward primer	GGAGCTTGAACGCGAAGAA
Reverse primer	GAGTCCATCGACTTGCTTCCAT

The mean $\pm$ SEM (n = 6) values of the gene/Actin ratio from the 1.5% agarose in TAE buffer gel image were plotted

using bar graphs. The computation of these values was done through Image-J. The representative snapshots of the pooled sample were also included.

Statistical analysis: To perform statistical analysis, Graphpad Prism version 9 was used. A nonparametric (one-way ANOVA) test was conducted, with statistical significance set at ($p < 0.05$).

RESULTS

Effect of PCOS induction on oestrus cycle: The vaginal smears of rats with polycystic ovarian cysts showed a dioestrus aphase with a predominant leukocyte cell type upon microscopic inspection.

Effect of clomiphene and carvedilol on reproductive hormones: As shown in Table 1, PCOS significantly decreased serum GnRH, LH, FSH, estradiol, and progesterone and increased serum prolactin compared with the control. These observed alterations were reversed by CLO, CAR, and CLO+CAR treatment, although animals treated with CLO+CAR exhibited better ameliorative effects. Similarly, CLO and CAR prevented PCOS-induced significant increase in AR, LH, and FSH gene expression compared with the control when used singly or combined (Figure 1).

Table 1: Effect of Carvedilol and Clomiphene on Reproductive Hormones

	Control	PCOS	PCOS+CLO	PCOS+CAR	PCOS+CLO+CAR
GnRH(ng/ml)	4.40 $\pm$ 0.4	5.03 $\pm$ 0.23a	4.02 $\pm$ 0.28b	4.12 $\pm$ 0.26b	4.30 $\pm$ 0.75b
FSH(mIU/ml)	0.46 $\pm$ 0.034	0.17 $\pm$ 0.011a	0.455 $\pm$ 0.03b	0.36 $\pm$ 0.039abc	0.32 $\pm$ 0.01abc
LH (mIU/ml)	2.14 $\pm$ 0.19	5.91 $\pm$ 0.57a	3.47 $\pm$ 0.35ab	3.13 $\pm$ 0.22ab	2.33 $\pm$ 0.38bcd
Prolactin (ng/ml)	2.68 $\pm$ 0.11	4.2 $\pm$ 0.21a	3.09 $\pm$ 0.26ab	3.76 $\pm$ 0.97abc	2.58 $\pm$ 0.01bcd
Testosterone (ng/ml)	0.24 $\pm$ 0.01	0.52 $\pm$ 0.03a	0.26 $\pm$ 0.01b	0.27 $\pm$ 0.02b	0.25 $\pm$ 0.01b
Estradiol (ng/ml)	3.25 $\pm$ 0.83	1.15 $\pm$ 0.93a	2.77 $\pm$ 0.17ab	3.01 $\pm$ 1.01ab	3.34 $\pm$ 0.38bcd
sProgesterone (ng/ml)	8.04 $\pm$ 0.11	4.65 $\pm$ 2.01a	8.12 $\pm$ 0.21b	8.18 $\pm$ 0.19b	8.01 $\pm$ 0.24b

The presented data represents the means $\pm$ SEM, where n = 6. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple post hoc test. The statistical analysis showed that there were significant differences ($p < 0.05$) between the control group and the groups with PCOS, PCOS+CLO, PCOS+CAR, and PCOS+CLO+CAR. PCOS refers to Polycystic Ovarian Syndrome, CLO refers to Clomiphene, CAR refers to carvedilol, GnRH refers to Gonadotropin Releasing Hormone, FSH refers to Follicle Stimulating Hormone, and LH refers to Luteinizing Hormone. The symbols a, b, c, and d indicate the levels of statistical significance compared to each group.

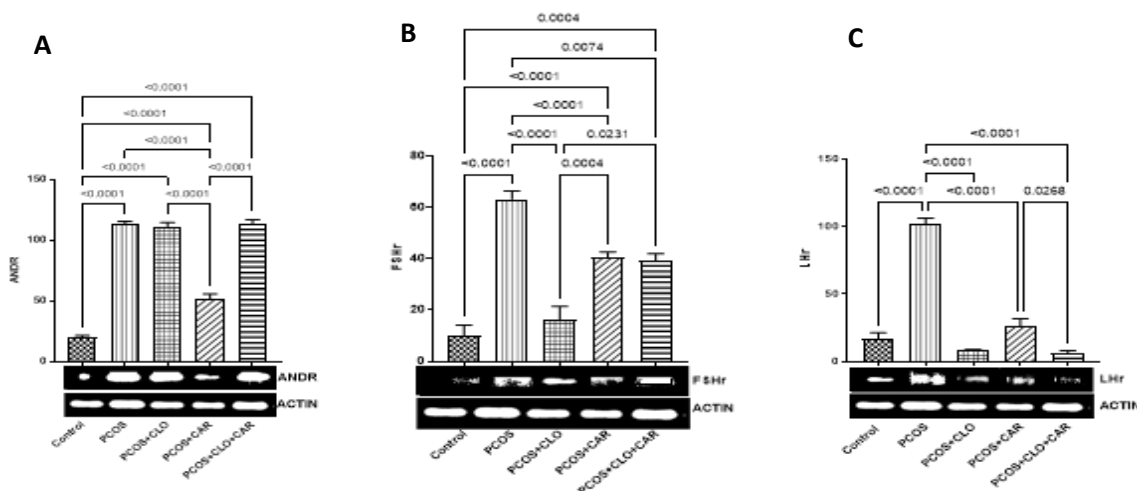


Figure 1:

Effect of Carvedilol and Clomiphene on Reproductive hormone receptors: The values for the quantified bands of the specified genes from each sample in the five groups were expressed as means $\pm$ SEM, where n = 6. The data was analysed using one-way analysis of variance (ANOVA), which was then followed by Tukey's multiple post hoc test. The gel image presented is representative of the pooled samples. Each bar graph shown represents the control-normalized relative expression of the Androgen receptor (AR), Follicle stimulating hormone receptor (FSHr), and Luteinizing hormone receptor (LHr) genes in relation to b-actin. The groups analyzed in this study include CONTROL, PCOS (polycystic ovarian syndrome), CLO (clomiphene), CAR (Carvedilol), PCOS+CLO+CAR).

Effect of Carvedilol and Clomiphene on Inflammatory markers: There was a significant increase in ovarian IL-1 β and NF- κ B gene expression in animals in the PCOS untreated group compared with the control. This observed difference was ameliorated by clomiphene, carvedilol, and clomiphene + carvedilol (Figure 2), although animals in the clomiphene + carvedilol treatment group exhibited a better ameliorative effect.

Effect of Carvedilol and Clomiphene on oxidative stress markers: PCOS significantly led to decreased ovarian catalase activities and downregulation of GSR, HMOX-1, and Nrf2 mRNA compared with the control (Figure 3). These observed differences were reversed in all the PCOS-treated groups, although clomiphene + carvedilol treatment was more effective in reversing the redox imbalance.

Effect of Carvedilol and Clomiphene on apoptotic markers: As shown in Figure 4, the expression of caspase 3 mRNA was upregulated in animals in the PCOS group. However, the observed upregulation was ameliorated by treatment with clomiphene and clomiphene + carvedilol.

Effect of Carvedilol and Clomiphene on histology of the ovary: The animals in the control group showed preserved ovarian outer cortex and an inner medulla. The cortical region is composed of follicles at varying degrees of maturity (Figure 5). The Graafian follicle is lined by cuboidal epithelium and contains a matured oocyte with a defined zona pellucida, zonal granulosa, antrum, and defined theca follicular cells. The medulla contained blood vessels and lymphatics. The corpus luteum appeared unremarkable. Unlike the animals in the control group, those in the PCOS showed a distorted ovarian outer cortex and an inner medulla. Furthermore, the animals treated with clomiphene and clomiphene with carvedilol showed preserved ovarian outer cortex and inner medulla and contained features consistent with the control. Although the animals treated with carvedilol also showed preserved ovarian outer cortex and inner medulla, their medulla contained severely congested blood vessels.

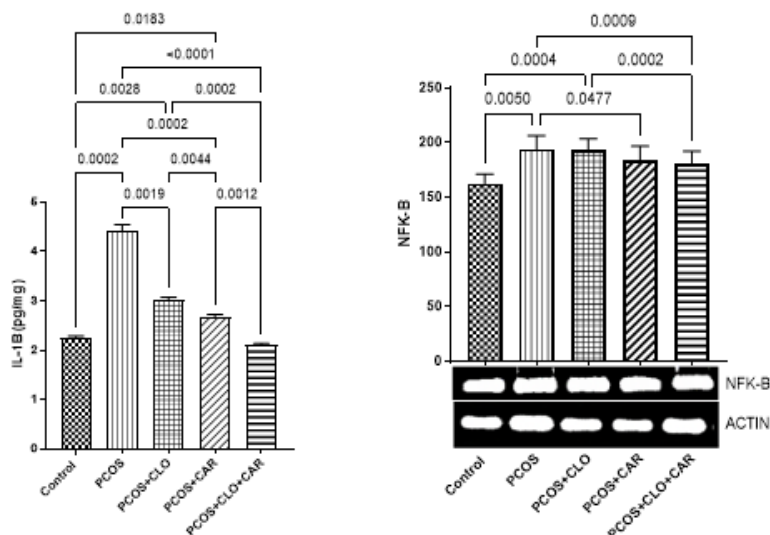


Figure 2: Effect of Carvedilol and Clomiphene on Inflammatory markers: Serum concentration of IL-1 β expressed as mean $\pm$ SEM, n=6. The values for the quantified bands of the specified genes from each sample in the five groups were expressed as means $\pm$ SEM, where n = 6. The data was analysed using one-way analysis of variance (ANOVA), which was then followed by Tukey's multiple post hoc test. The gel image presented is representative of the pooled samples. Each bar graph shown represents the control-normalized relative expression of the NFKb gene in relation to b-actin. The groups analyzed in this study include CONTROL, PCOS (polycystic ovarian syndrome), CLO (clomiphene), CAR (Carvedilol), PCOS+CLO+CAR)

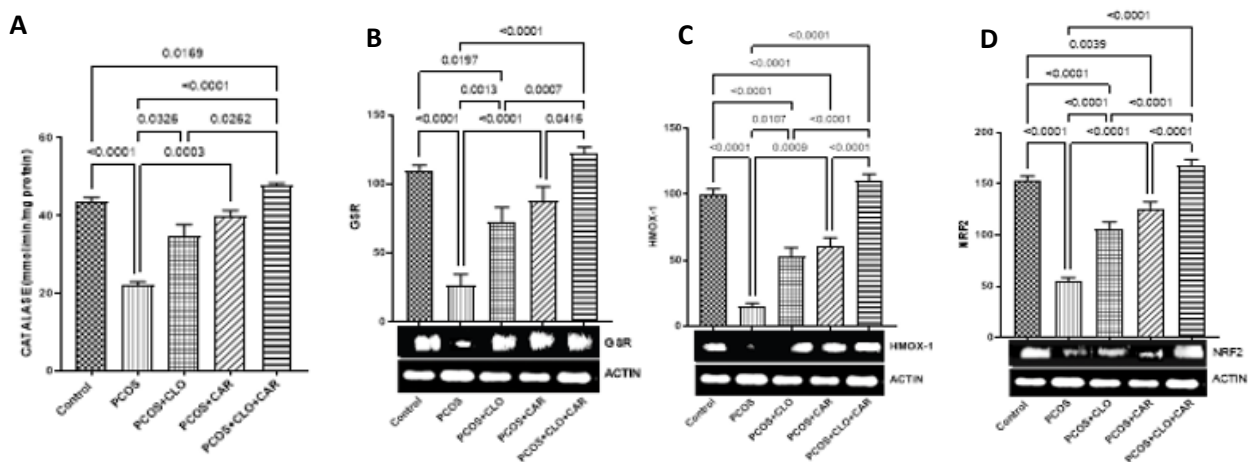
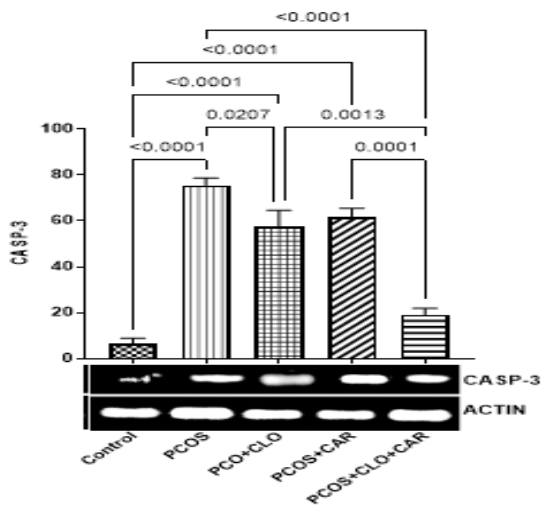
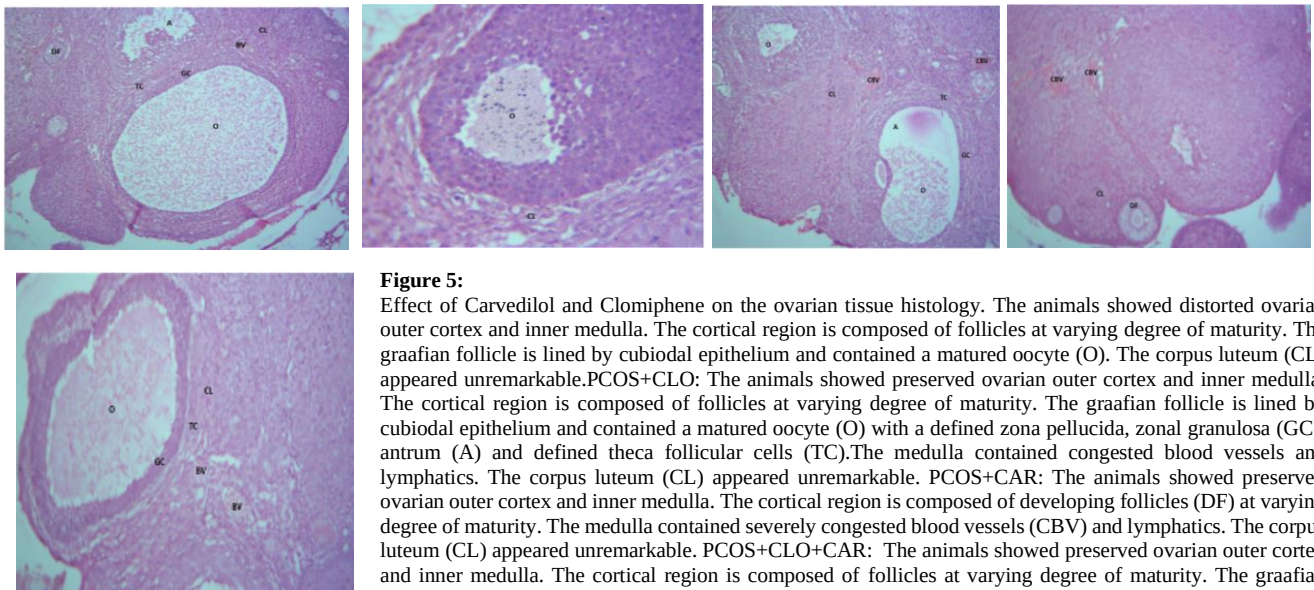


Figure 3: Carvedilol and Clomiphene synergistically improved the antioxidant status of PCOS rats: Serum concentration of Catalase expressed as mean $\pm$ SEM, n=6. The values for the quantified bands of the specified genes from each sample in the five groups were expressed as means $\pm$ SEM, where n = 6. The data was analysed using one-way analysis of variance (ANOVA), which was then followed by Tukey's multiple post hoc test. The gel image presented is representative of the pooled samples. Each bar graph shown represents the control-normalized relative expression of the GSR, HMOX-1, NRF2, gene in relation to b-actin. The groups analyzed in this study include CONTROL, PCOS (polycystic ovarian syndrome), CLO (clomiphene), CAR (Carvedilol), PCOS+CLO+CAR)

Carvedilol and Clomiphene prevents PCOS-induced oxido-inflammatory response

**Figure 4:**

Carvedilol and Clomiphene demonstrates synergistic anti-apoptotic properties: The values for the quantified bands of the specified genes from each sample in the five groups were expressed as means $\pm$ SEM, where n = 6. The data was analysed using one-way analysis of variance (ANOVA), which was then followed by Tukey's multiple post hoc test. The gel image presented is representative of the pooled samples. Each bar graph shown represents the control-normalized relative expression of the Caspase 3 gene in relation to b-actin. The groups analyzed in this study include CONTROL, PCOS (polycystic ovarian syndrome), CLO (clomiphene), CAR (Carvedilol), PCOS+CLO+CAR

**Figure 5:**

Effect of Carvedilol and Clomiphene on the ovarian tissue histology. The animals showed distorted ovarian outer cortex and inner medulla. The cortical region is composed of follicles at varying degree of maturity. The graafian follicle is lined by cuboidal epithelium and contained a matured oocyte (O). The corpus luteum (CL) appeared unremarkable. PCOS+CLO: The animals showed preserved ovarian outer cortex and inner medulla. The cortical region is composed of follicles at varying degree of maturity. The graafian follicle is lined by cuboidal epithelium and contained a matured oocyte (O) with a defined zona pellucida, zonal granulosa (GC), antrum (A) and defined theca follicular cells (TC). The medulla contained congested blood vessels and lymphatics. The corpus luteum (CL) appeared unremarkable. PCOS+CAR: The animals showed preserved ovarian outer cortex and inner medulla. The cortical region is composed of developing follicles (DF) at varying degree of maturity. The medulla contained severely congested blood vessels (CBV) and lymphatics. The corpus luteum (CL) appeared unremarkable. PCOS+CLO+CAR: The animals showed preserved ovarian outer cortex and inner medulla. The cortical region is composed of follicles at varying degree of maturity. The graafian follicle is lined by cuboidal epithelium and contained a matured oocyte (O) with a defined zona pellucida, zonal granulosa (GC), antrum (A) and defined theca follicular cells (TC). The medulla contained mildly congested blood vessels and lymphatics. The corpus luteum (CL) appeared unremarkable.

DISCUSSION

This study reported for the first time that clomiphene and carvedilol synergistically decrease oxidative stress, inflammatory, and apoptotic biomarkers in Letrozole-induced PCOS in female rats. Interestingly, these ameliorative effects were consistent with the maintenance of hormonal balance, expression of hormone receptors, and significant improvement in the histology of the ovary.

The findings from this study that PCOS disrupts endocrine functions by increasing GnRH, LH, testosterone, and prolactin production and decreasing FSH, estrogen, and progesterone release is similar to previous studies (Dennett and Simon, 2015; Oyeboji *et al.*, 2018; Mikhael *et al.*, 2019). An increase in testosterone (hyperandrogenism) is the hallmark of PCOS (Ashraf *et al.*, 2019). An increase in testosterone disrupts folliculogenesis by stimulating the production of primordial follicles and elevating the number of small antral follicles (Nisenblat and Norman, 2009). Ideally, GnRH is released from the hypothalamus and acts on the pituitary gland to secrete gonadotropins (LH and FSH). While LH acts majorly on the ovarian theca cells to

stimulate the production of androgens, FSH acts on the ovarian granulosa cells to act on the androgens produced from the theca cells and converts it into estrogens (majorly estradiol) which is the major female sex hormone that stimulates the development of follicles (Nisenblat and Norman, 2009). Unfortunately, PCOS is characterized by an increase in GnRH which favors the production of LH and not FSH (Fauser *et al.*, 1991; Dennett and Simon, 2015), leading to the classical increase in LH/FSH ratio and, consequently increase in circulating testosterone. Due to the increase in LH secretion, the growth of the follicles in the theca cells will be arrested mostly in the preantral and antral stages leading to theca cells hyperplasia and the formation of cyst-like structure on the ovary (Abbott *et al.*, 2005; Dennett and Simon, 2015). Also, the increase in testosterone has been linked with hyperinsulinemia, suggesting multiple pathways for PCOS-induced hyperandrogenism. PCOS has been shown to increase insulin production from the pancreas, which can mimic LH's tropic action on the theca cells (Wu *et al.*, 2014).

Furthermore, the observed decrease in progesterone indicates a distorted menstrual cycle known as an

anovulatory cycle. The hypothalamic-pituitary-ovarian (HPO) axis promotes ovulation by selecting a dominant follicle and priming the endometrium for implantation (Cha *et al.*, 2012). Once the ovary is released during ovulation, the empty follicle sac will start secreting progesterone to inhibit the hypothalamic and pituitary glands, thereby reducing estrogen production and increasing progesterone. Furthermore, the observed hyperprolactinemia from this study disagreed with the study of Yang *et al.*, 2020. This could result from decreased dopamine secretion (Chaudhari *et al.*, 2018) negatively correlated with prolactin secretion (Gragnoli *et al.*, 2016). It is tempting to conclude that the associated infertility with PCOS results from PCOS-induced hyperprolactinemia since hyperprolactinemia has been reported to cause infertility (Odetayo and Olayaki, 2022). This study agrees with previous studies that clomiphene and carvedilol can individually ameliorate PCOS-induced hormonal imbalance (Rezyamfar *et al.*, 2016; Takasaki *et al.*, 2018). However, findings from this study also showed that the combination of both drugs is also effective in treating PCOS-induced hormonal imbalance.

In addition, the findings from this study that PCOS caused overexpression of AR, FSHR, and LHR supports our claim that PCOS is an endocrine disruptor and clomiphene and carvedilol synergistically ameliorated PCOS-induced hormonal imbalance. Overexpression of AR and LHR indicates an increase in testosterone and LH synthesis, while that of FSHR could result from decreased FSH synthesis. FSH-induced decrease in FSHR mRNA has previously been reported (Themmen *et al.*, 1991; Griswold *et al.*, 2001). In fact, an increase in FSHR expression has been reported in hypophysectomized rats (Maguire *et al.*, 1997). Griswold *et al.*, 2001 observed a 50% decrease in FSHR mRNA levels after 5 hours of FSH stimulation. This decrease in FSHR mRNA could be due to the ability of FSH to decrease the expression of upstream stimulatory factor 1 (USF1) and increase the accumulation of inhibitor of DNA binding/differentiation (ID)-2 repressor protein to distort transcription of FSHR (Viswanathan *et al.*, 2009). Also, this study reported that PCOS induced inflammatory response evidenced by the increase in IL-1 β and NF- κ B, which agreed with a previous study that chronic inflammation is one of the pathogenesis of PCOS (Rudnicka *et al.*, 2021). NF- κ B is activated via two main pathways, canonical and noncanonical (Liu *et al.*, 2017). The canonical pathway involves responding to different stimuli, while the noncanonical response is to a specific group of stimuli. NF- κ B is a major regulator of inflammatory responses. Its overexpression is a hallmark of chronic inflammatory diseases (Liu *et al.*, 2017; Akhigbe *et al.*, 2021) since it stimulates the release of pro-inflammatory markers such as IL-1 β (Afolabi *et al.*, 2022a). Since overexpression of NF- κ B has been implicated in various inflammatory diseases, targeting NF- κ B signaling could open a new therapeutic window in treating PCOS complications. The findings from this study that clomiphene and carvedilol synergistically ameliorate PCOS-induced overexpression of NF- κ B is novel and suggests their effectiveness in treating inflammatory diseases.

Oxidative stress has been implicated in PCOS-induced ovarian damage (Mohammadi, 2019). It is due to a PCOS-led imbalance between pro-oxidants and antioxidants, resulting in ovarian damage. Furthermore, PCOS is

associated with excessive adipokines generation (Lin *et al.*, 2021), resulting in cytokine storm which aggravates oxidative damage leading to structural and functional cellular impairment and cell death (Akhigbe *et al.*, 2021; Adeyemi *et al.*, 2022). Oxidative stress also leads to the generation of reactive oxygen species (ROS), which can destroy the ovarian follicles because of the high content of polyunsaturated fatty acids in the cells (Afolabi *et al.*, 2022b). The findings from this study that clomiphene and carvedilol upregulate the gene expression of Nrf2, HO-1, and GSR and serum catalase suggests their antioxidant capacity in PCOS-induced oxidative stress. Nrf2 is a cytoprotective factor that regulates the expression of proteins responsible for protecting the cell against oxidative stress, inflammation, and apoptosis (Loboda *et al.*, 2016). Nrf2 regulates the expression of different cytoprotective genes, such as the ones that regulate the synthesis of glutathione and HO-1, a potent antioxidant. Apart from being an antioxidant, HO-1 also regulates different important biological activities such as inflammation, apoptosis, cell proliferation, and angiogenesis (Loboda *et al.*, 2016). Putting these pieces of information together, it is tempting to conclude that combining clomiphene and carvedilol synergistically reverses PCOS-induced female infertility by acting as antioxidants via Nrf2/HO-1-dependent mechanism.

The observed PCOS-induced oxidative stress accompanied by an increase in the inflammatory response in the ovaries explains the observed PCOS-induced increase in caspase 3 gene expression. Since it has been shown that oxidative stress can stimulate inflammatory response and vice versa, PCOS could possibly lead to overwhelming oxido-inflammatory damage to the ovary. PCOS possibly induces the generation of free radicals, which stimulate caspase 3 by stimulating the intrinsic apoptotic pathway (Aitken and Koppers, 2010; Hamed *et al.*, 2022). Also, the pro-apoptotic pathway may be activated by NF- κ B by enhancing the phosphorylation of c-Jun and caspase 3 activations (Li *et al.*, 2017). Findings from this study showed that clomiphene and carvedilol reduced apoptosis in animals with PCOS, which could result from their anti-inflammatory and antioxidant capacity since both inflammation and oxidative stress form a vicious cycle with apoptosis (Akhigbe *et al.*, 2021). Also, clomiphene and carvedilol could activate peroxisome proliferator-activated receptor- γ (PPAR γ), which might inhibit p65 NF- κ B (involved in inflammatory response), and upregulates I κ B to inhibit NF- κ B, thereby halting inflammatory response (Edwards and O'Flaherty, 2008; Zayed *et al.*, 2018; Hassanein *et al.*, 2020). PPAR γ activation can also stimulate Nrf2 expression, stimulating the release of HO-1, thereby conferring cellular protection (Edwards and O'Flaherty, 2008; Zayed *et al.*, 2018; Hassanein *et al.*, 2020). Also, this study showed that PCOS distorts the histology of the ovary, evidenced by a distorted ovarian outer cortex and an inner medulla. This finding is in tandem with the study of Wang *et al.*, 2012, who also reported morphologic differences and organelle structure changes in the ovaries of animals with PCOS.

In conclusion, this study is an extension of existing data on the pathophysiological processes of PCOS. The present study also explored the therapeutic activities of clomiphene and carvedilol in PCOS-induced ovarian dysfunction. This

study demonstrated for the first time that clomiphene and carvedilol protect the ovary by ameliorating the effect of PCOS-induced oxido-inflammatory damage and apoptosis via the stimulation of Nrf2/HMOX-1 and downregulation of NfκB signaling. This suggests that combining clomiphene and carvedilol is a novel therapeutic candidate to be explored in managing PCOS. However, clinical studies are strongly recommended to validate the protective potentials of clomiphene and carvedilol in PCOS patients.

Declarations

Ethical Approval

The animals were carefully handled as stated by the National Institute of Health (NIH), and ARRIVE guidelines for reporting experimental findings were strictly followed. The experimental research protocol was designed according to the National Research Council's guidelines for the Care and Use of Laboratory Animals, and ethical approval was obtained from the Institutional Ethical Review Committee.

Competing Interest

The authors have no conflicts of interest to declare

Authors' contributions

AAJ, AOO, OAF and OLA conceived and designed the study; AAJ, AOO, OAI, OST, OAF, BAM, and OLA carried out the experiments. AAJ, AOO, OAI, OST, OAF, BAM, and OLA contributed reagents and analytical kits. AAJ and OAF analyzed and interpreted data. OAF drafted the manuscript. AAJ, AOO, OAI, OST, OAF, BAM and OLA read and approved the final manuscript

REFERENCES

- Abbott, D.H., Barnett, D.K., Bruns, C.M., Dumesic, D.A. (2005). Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome?. *Human reproduction update*; 11(4), 357–374.
- Adeyemi, D.H., Odetayo, A.F., Hamed, M.A., Akhigbe, R.E. (2022). Impact of COVID 19 on erectile function. The aging male: the official journal of the International Society for the Study of the Aging Male; 25(1), 202–216.
- Afolabi, O.A., Anyogu, D.C., Hamed, M.A., Odetayo, A.F., Adeyemi, D.H., Akhigbe, R. E. (2022a). Glutamine prevents upregulation of NF-κB signaling and caspase 3 activation in ischaemia/reperfusion-induced testicular damage: An animal model. *Biomedicine and pharmacotherapy*; 150, 113056.
- Afolabi, O.A., Hamed, M.A., Anyogu, D.C., Adeyemi, D.H., Odetayo, A.F., Akhigbe, R.E. (2022b). Atorvastatin-mediated downregulation of VCAM-1 and XO/UA/caspase 3 signaling averts oxidative damage and apoptosis induced by ovarian ischaemia/reperfusion injury. *Redox report: communications in free radical research*; 27(1), 212–220.
- Aikoye, S.A., Jafferany, M., Osuagwu, V., Plath, D.L. (2019). The Man Who Saw Things on Carvedilol. *The Tokai Journal of Experimental and Clinical Medicine*; 44(2), 29–30.
- Aitken, R.J., Koppers, A.J. (2010). Apoptosis and DNA damage in human spermatozoa. *Asian Journal of Andrology*; 13(1), 36–42.
- Akhigbe, R.E., Hamed, M.A., Odetayo, A.F., Akhigbe, T.M., Ajayi, A.F., Ajibogun, F. (2021). Omega-3 fatty acid rescues ischaemia/perfusion-induced testicular and sperm damage via modulation of lactate transport and xanthine oxidase/uric acid signaling. *Biomedicine and pharmacotherapy*; 142, 111975.
- Akintoye, O.O., Ajibare, A.J., Omotuyi, I.O. (2023). Virgin coconut oil reverses behavioral phenotypes of letrozole-model of PCOS in Wistar rats via modulation of NRF2 upregulation. *Journal of Taibah University Medical Sciences*.
- Al Khalifah, R.A., Florez, I.D., Zoratti, M.J., Dennis, B., Thabane, L., Bassilious, E. (2021). Efficacy of Treatments for Polycystic Ovarian Syndrome Management in Adolescents. *Journal of the Endocrine Society*; 5(1).
- Alesi, S., Ee, C., Moran, L.J., Rao, V., Mousa, A. (2022). Nutritional Supplements and Complementary Therapies in Polycystic Ovary Syndrome. *Advances in Nutrition*; 13(4), 1243–1266.
- Ashraf, S., Nabi, M., Rasool, S., Ul, A., Rashid, F., Amin, S. (2019). Hyperandrogenism in polycystic ovarian syndrome and role of CYP gene variants: a review. *Egyptian Journal of Medical Human Genetics*; 20(1).
- Bakr, A.G., Hassanein, E.H.M., Ali, F.E.M., El-Shoura, E.A.M. (2022). Combined apocynin and carvedilol protect against cadmium-induced testicular damage via modulation of inflammatory response and redox-sensitive pathways. *Life Sciences*; 311, 121152.
- Cha, J., Sun, X., Dey, S.K. (2012). Mechanisms of implantation: strategies for successful pregnancy. *Nature medicine*; 18(12), 1754–1767.
- Chaudhari, N., Dawalbhakta, M., Nampoothiri, L. (2018). GnRH dysregulation in polycystic ovarian syndrome (PCOS) is a manifestation of an altered neurotransmitter profile. *Reproductive biology and endocrinology*; 16(1), 37.
- Chaudhuri, A. (2023). Polycystic ovary syndrome: Causes, symptoms, pathophysiology, and remedies. *Obesity Medicine*; 39, 100480.
- Chouhan, A.S. (2022). Advance Research on Polycystic Ovary Syndrome (PCOS). *Journal of Womens Healthcare and Midwifery Research*; 1–5.
- Cunha, A., Póvoa, A.M. (2021). Infertility management in women with polycystic ovary syndrome: a review. *Porto Biomedical Journal*; 6(1), e116.
- Dabadghao, P. (2019). Polycystic ovary syndrome in adolescents. *Best Practice & Research Clinical Endocrinology & Metabolism*; 33(3), 101272.
- Dennett, C.C., Simon, J. (2015). The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. *Diabetes spectrum: a publication of the American Diabetes Association*; 28(2), 116–120.
- Edwards, I.J., O'Flaherty, J.T. (2008). Omega-3 Fatty Acids and PPARγ in Cancer. *PPAR Research*; 1–14.
- Fausser, B.C., Pache, T.D., Lamberts, S.W., Hop, W.C., de Jong, F.H., Dahl, K.D. (1991). Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease. *The Journal of clinical endocrinology and metabolism*; 73(4), 811–817.
- Gagnoli, C., Reeves, G.M., Reazer, J., Postolache, T.T. (2016). Dopamine-prolactin pathway potentially contributes to the schizophrenia and type 2 diabetes comorbidity. *Translational psychiatry*; 6(4), e785.
- Griswold, M.D., Kim, J.S., Tribley, W.A. (2001). Mechanisms involved in the homologous down-regulation of transcription of the follicle-stimulating hormone receptor gene in Sertoli cells. *Molecular and cellular endocrinology*; 173(1-2), 95–107.
- Hamed, M.A., Akhigbe, R.E., Aremu, A.O., Odetayo, A.F. (2022). Zinc normalizes hepatic lipid handling via modulation of ADA/XO/UA pathway and caspase 3

- signaling in highly active antiretroviral therapy-treated Wistar rats. *Chemico-biological interactions*; 368, 110233.
- Hassanein, E.H.M., Sayed, A.M., Hussein, O.E., Mahmoud, A.M. (2020). Coumarins as Modulators of the Keap1/Nrf2/ARE Signaling Pathway. *Oxidative Medicine and Cellular Longevity*; 1–25.
- Joham, A.E., Norman, R.J., Stener-Victorin, E., Legro, R.S., Franks, S., Moran, L.J., Boyle, J., and Teede, H.J. (2022). Polycystic ovary syndrome. *The Lancet Diabetes & Endocrinology*; 10(9), 668–680.
- Li, J., Liu, Y., Duan, P., Yu, R., Gu, Z., Li, L., LIU, Z., Su, L. (2017). NF- κ B regulates HSF1 and cJun activation in heat stress-induced intestinal epithelial cell apoptosis. *Molecular Medicine Reports*; 17: 3388–3396.
- Lin, K., Sun, X., Wang, X., Wang, H., Chen, X. (2021). Circulating Adipokine Levels in Nonobese Women with Polycystic Ovary Syndrome and in Nonobese Control Women: A Systematic Review and Meta-Analysis. *Frontiers in endocrinology*; 11, 537809.
- Liu, T., Zhang, L., Joo, D., Sun, S.C. (2017). NF- κ B signaling in inflammation. *Signal transduction and targeted therapy*; 2, 17023.
- Liu, T., Zhang, L., Joo, D., Sun, S.C. (2017). NF- κ B signaling in inflammation. *Signal transduction and targeted therapy*; 2, 17023.
- Loboda, A., Damulewicz, M., Pyza, E., Jozkowicz, A., Dulak, J. (2016). Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cellular and molecular life sciences*; 73(17), 3221–3247.
- Maguire, S.M., Tribbley, W.A., Griswold, M.D. (1997). Follicle-stimulating hormone (FSH) regulates the expression of FSH receptor messenger ribonucleic acid in cultured Sertoli cells and in hypophysectomized rat testis. *Biology of reproduction*; 56(5), 1106–1111.
- Marouf, B.H., Ismael, D.O., Hassan, A.H., Ali, O.J. (2022). Therapeutic Effects of Silibinin Against Polycystic Ovary Syndrome Induced by Letrozole in Rats via Its Potential Anti-Inflammatory and Anti-Oxidant Activities. *Journal of Inflammation Research*; 15, 5185–5199.
- Mikhael, S., Punjala-Patel, A., Gavrilova-Jordan, L. (2019). Hypothalamic-Pituitary-Ovarian Axis Disorders Impacting Female Fertility. *Biomedicines*; 7(1), 5.
- Mohammadi, M. (2019). Oxidative Stress and Polycystic Ovary Syndrome: A Brief Review. *International journal of preventive medicine*; 10, 86.
- Nisenblat, V., Norman, R.J. (2009). Androgens and polycystic ovary syndrome. *Current opinion in endocrinology, diabetes, and obesity*; 16(3), 224–231.
- Odetayo, A.F., Olayaki, L.A. (2022). Effect of bisphenol F on sexual performance and quality of offspring in Male Wistar rats. *Ecotoxicology and environmental safety*; 244, 114079.
- Oyebanji, O., Asaolu, M., Amonimo, E. (2018). Hormonal Imbalance in Polycystic Ovarian Syndrome (PCOS) in Teaching Hospitals in Ekiti State, Nigeria. *Open Journal of Obstetrics and Gynecology*; 8, 1456–1464.
- Rezvandar, M.A., Saeedi, S., Mansoori, P., Saadat, S., Goosheh, M., Shojaei Saadi, H.A., Baeri, M., Abdollahi, M. (2016). Dual targeting of TNF- α and free radical toxic stress as a promising strategy to manage experimental polycystic ovary. *Pharmaceutical biology*; 54(1), 80–90.
- Rudnicka, E., Suchta, K., Grymowicz, M., Calik-Ksepka, A., Smolarczyk, K., Duszewska, A.M., Smolarczyk, R., Meczekalski, B. (2021). Chronic Low Grade Inflammation in Pathogenesis of PCOS. *International journal of molecular sciences*; 22(7), 3789.
- Sun, S.C., Chang, J.H., Jin, J. (2013). Regulation of nuclear factor- κ B in autoimmunity. *Trends in immunology*; 34(6), 282–289.
- Takasaki, A., Tamura, I., Okada-Hayashi, M., Orita, T., Tanabe, M., Maruyama, S., Shimamura, K., Morioka, H. (2018). Usefulness of intermittent clomiphene citrate treatment for women with polycystic ovarian syndrome that is resistant to standard clomiphene citrate treatment. *Reproductive medicine and biology*; 17(4), 454–458.
- Themmen, A.P., Blok, L.J., Post, M., Baarends, W.M., Hoogerbrugge, J.W., Parmentier, M., Vassart, G., Grootegeed, J.A. (1991). Follicle-stimulating hormone receptor down-regulation involves a cAMP-dependent post-transcriptional decrease of receptor mRNA expression. *Molecular and cellular endocrinology*; 78(3), R7–R13.
- Viswanathan, P., Wood, M.A., Walker, W.H. (2009). Follicle-stimulating hormone (FSH) transiently blocks FSH receptor transcription by increasing inhibitor of deoxyribonucleic acid binding/differentiation-2 and decreasing upstream stimulatory factor expression in rat Sertoli cells. *Endocrinology*; 150(8), 3783–3791.
- Wahab, O.A., Princely, A.C., Oluwadamilare, A.A., Ore-Oluwapo, D.O., Blessing, A.O., Alfred, E.F. (2019). Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. *JBRA assisted reproduction*; 23(4), 336–343.
- Wang, F., Yu, B., Yang, W., Liu, J., Lu, J., Xia, X. (2012). Polycystic ovary syndrome resembling histopathological alterations in ovaries from prenatal androgenized female rats. *Journal of ovarian research*; 5(1), 15.
- Wu, S., Divall, S., Nwaopara, A., Radovick, S., Wondisford, F., Ko, C., Wolfe, A. (2014). Obesity-induced infertility and hyperandrogenism are corrected by deletion of the insulin receptor in the ovarian theca cell. *Diabetes*; 63(4), 1270–1282.
- Xiong, L., Xie, J., Song, C., Liu, J., Zheng, J., Liu, C., Zhang, X., Li, P., Wang, F. (2015). The Activation of Nrf2 and Its Downstream Regulated Genes Mediates the Antioxidative Activities of Xueshuan Xinmaining Tablet in Human Umbilical Vein Endothelial Cells. *Evidence-based complementary and alternative medicine: eCAM*; 187265.
- Xu, Y., Zhu, H., Li, W., Chen, D., Xu, Y., Xu, A., Ye, D. (2022). Targeting adipokines in polycystic ovary syndrome and related metabolic disorders: from experimental insights to clinical studies. *Pharmacology and Therapeutics*; 240, 108284.
- Yang, H., Di, J., Pan, J., Yu, R., Teng, Y., Cai, Z., Deng, X. (2020). The Association Between Prolactin and Metabolic Parameters in PCOS Women: A Retrospective Analysis. *Frontiers in endocrinology*; 11, 263.
- Yeon Lee, J., Baw, C.K., Gupta, S., Aziz, N., Agarwal, A. (2010). Role of Oxidative Stress in Polycystic Ovary Syndrome. *Current Women's Health Reviews*; 6(2), 96–107.
- Zayed, E.A., AinShoka, A.A., El Shazly, K.A., Abd El Latif, H.A. (2018). Improvement of insulin resistance via increase of GLUT4 and PPAR γ in metabolic syndrome-induced rats treated with omega-3 fatty acid or l-carnitine. *Journal of Biochemical and Molecular Toxicology*; e22218.

Full length Research Article

Ursolic Acid Ameliorates Vascular Oxidative Stress and Upregulates Endothelial Nitric Oxide Synthase Gene in Male Wistar Rats with High-carbohydrate High-fat Diet-induced Metabolic Syndrome

***Omodara, O.O.¹, Kawu, M.U.², Bako, I.G.³, Isa, A.S.³, Mhya, H.D.⁴, Ali, Z.¹.**

Departments of ¹Human Physiology and ⁴Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

²Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

³Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Summary: The development of cardiovascular diseases and type 2 diabetes is preceded by the risk factors of metabolic syndrome (MS) which are often induced by high-carbohydrate high-fat diet (HCHFD) together with sedentary lifestyle. These risk factors are associated with vascular dysfunction. Our previous study has shown that ursolic acid (UA) prevents the development of these risk factors of MS induced by HCHFD, but the potential mechanism involved in the amelioration of vascular oxidative stress induced by HCHFD has not been explained. This study investigated the mechanism by which dietary UA supplementation improves vascular oxidative stress in male Wistar rats fed a HCHFD. Twenty (20) male Wistar rats were randomly divided into 4 groups (n =5): 1- normal diet (ND) + distilled water (DW); 2 – ND+UA; 3 – HCHFD+DW; 4 – HCHFD+UA. HCHFD was formulated in-house. The animals were fed their respective diets daily for 20 weeks. The drinking water of animals fed a HCHFD was augmented with 20% fructose. 250 mg/kg body weight of ursolic acid was administered orally to UA-treated groups for the last 8 weeks of the study. Body mass index (BMI) and abdominal circumference were evaluated; serum insulin and nitric oxide were assessed by using enzyme-linked immunosorbent assay kits; and insulin resistance was determined using the homeostatic model assessment for insulin resistance (HOMA-IR). Aortic antioxidant enzymes and reactive oxygen species were evaluated. Aorta and adipose tissues' endothelial nitric oxide synthase (eNOS) was evaluated using real-time polymerase chain reaction technique. There was a significantly (P<0.05) lowered BMI percentage increase in the HCHFD+UA-fed animals compared to the HCHFD+DW-fed animals. In the HCHFD+UA-fed animals, serum insulin and HOMA-IR were significantly (P<0.05) decreased compared to the HCHFD+DW-fed animals. Serum nitric oxide was significantly (P<0.05) increased in HCHFD+UA-fed animals compared to the HCHFD+DW-fed animals. In HCHFD+UA-fed animals, aorta superoxide dismutase, catalase and glutathione were significantly (P<0.05) increased, compared to the HCHFD+DW-fed animals. Aorta reactive oxygen species was significantly (P<0.05) decreased in HCHFD+UA-fed animals compared to the HCHFD+DW-fed animals. Both aorta and adipose tissue eNOS mRNA level was significantly (P<0.05) more expressed in the HCHFD+UA-fed animals compared to the HCHFD+DW-fed animals. Findings from this study showed that ursolic acid supplementation ameliorates vascular oxidative stress and upregulates eNOS gene in male Wistar rats with high-carbohydrate high-fat diet (HCHFD)-induced metabolic syndrome.

Keywords: Ursolic Acid, Metabolic Syndrome, High-carbohydrate high-fat diet, Vascular oxidative stress, Endothelial dysfunction, Endothelial nitric oxide synthase gene.

*Authors for correspondence: tosomod2015@gmail.com, Tel: +234-8038580331

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INTRODUCTION

Metabolic syndrome (MS) has become a growing epidemic with over one billion people in the world affected (Tran *et al.*, 2020). Obesity is a component of MS. According to recent data, more than 1 billion people in the world are obese, including 650 million adults, 340 million

adolescents, and 39 million children, which will cause about 167 million people to become ill by 2025 (WHO, 2022). The components/diagnostic criteria of MS, which are central obesity, elevated blood pressure, impaired glucose tolerance, insulin resistance, and dyslipidemia, are risk factors for the development of cardiovascular disease

(CVD) and type 2 diabetes (T2D) (O'Neill and O'Driscoll, 2015; Tune *et al.*, 2017). These risk factors, which are often induced by high-carbohydrate high-fat diet (HCHFD) (Omodara *et al.*, 2022) together with sedentary lifestyle, are associated with vascular dysfunction (Tran *et al.*, 2020). The maintenance of normal vascular homeostasis depends essentially on healthy endothelial cell layer. In normal physiology, the endothelium produces several paracrine factors that regulate vascular tone, limit expression of proinflammatory molecules, inhibit platelet aggregation, promote fibrinolysis, and limit smooth muscle proliferation (Meyers and Gokce, 2007; Lee *et al.*, 2017). Endothelial-derived nitric oxide (NO) is the principal driver of the vasodilatory process. eNOS is mostly expressed in endothelial cells, it synthesizes NO in a pulsatile manner and its activity is markedly increased when intracellular Ca^{2+} rises. With respect to the regulation of vascular tone, one of the most important physiological stimuli for eNOS activation is the frictional force of the flowing blood exerted on the endothelial cell surface, known as the fluid shear stress (Shaul, 2002; Lam *et al.*, 2006). This stimulus does not produce sustained increases in intracellular Ca^{2+} (Forstermann and Sessa, 2012). When exposed to the increased shear stress, healthy endothelium responds with increased NO production owing to the phosphorylation of eNOS (Corson *et al.*, 1996). Once synthesized, NO diffuses to VSMC and induces endothelium-dependent, flow-mediated vasodilatation to accommodate the increased flow (Rapoport *et al.*, 1983; Forstermann *et al.*, 1986). NO molecules vasodilate the local vascular smooth muscles by stimulating guanylyl cyclase and increasing production of cyclic guanosine monophosphate (cGMP) (Forstermann and Sessa, 2012). In addition to its vasodilatory effects, NO also acts as a potent inhibitor of platelet aggregation and adhesion, interferes with leukocyte adhesion, and inhibits proliferation of vascular smooth muscles (Forstermann and Sessa, 2012; Lee *et al.*, 2017). Disruption of these processes results in unhealthy endothelium which leads to vascular dysfunction.

Previous data have shown vascular dysfunction in conduit and small arteries in patients with metabolic syndrome (Schillaci *et al.*, 2005; Greenstein *et al.*, 2009). Dysfunctional resistance arteries play a key role in the development of hypertension and is mediated, at least in part, by oxidative stress (Schiffrin and Touyz, 2004). In the vascular system, reactive oxygen species (ROS) are produced by different cell types, such as endothelial cells, vascular smooth muscle cells, and inflammatory cells infiltrating the perivascular tissue (Di *et al.*, 1999; De Ciuceis *et al.*, 2005). NADPH oxidase represents the major source of ROS in the vasculature (Schiffrin, 2008). In addition, the enzyme endothelial NO synthase (eNOS), which normally is "coupled" and produces NO, under some conditions, such as in the presence of excess oxidative stress or decreased tetrahydrobiopterin, is "uncoupled" and generates superoxide ($\cdot\text{O}_2^-$). This leads to the production of reactive nitrogen species, such as peroxynitrite, as a result of the action of $\cdot\text{O}_2^-$ on NO. Thus, in the vascular system, oxidative stress from different sources decreases NO bioavailability, thereby promoting endothelial dysfunction, vasoconstriction, remodeling, and enhanced systemic vascular resistance, leading to blood pressure increase (Touyz and Schiffrin, 2004; Schiffrin, 2008).

Markers of oxidative stress have been studied as a means to assess endothelial injury and dysfunction because reduction in NO bioavailability is often due to increased pro-oxidant stress. Glutathione maintains thiol groups of biomolecules in their reduced state and prevents peroxidation of membrane lipids (Molyneux *et al.*, 2002). Similarly, high cysteine levels are indicative of increased oxidative stress. Glutathione is also involved in transportation of NO (Bohlen *et al.*, 2009). Previous studies have found associations between increased oxidative stress, measured as lower glutathione and/or higher cysteine levels and flow-mediated dilation (FMD), microvascular vasodilator function, arterial stiffness, and arterial thickness (Ashfaq *et al.*, 2008; Dhawan *et al.*, 2011; Patel *et al.*, 2011; Mkhwanazi *et al.*, 2014).

Insulin resistance is marked by hyperinsulinemia and hyperglycemia. Under normal conditions, insulin enhances the vasodilatory action of NO and increases its production, however, display paradoxical vasoconstriction when exposed to high level of insulin. In patients with insulin-dependent diabetes mellitus, the serum insulin concentration is inversely correlated with endothelium-dependent vasodilation (EDV) (Johnstone *et al.*, 1993). Furthermore, insulin administration itself impairs endothelial function (Arcaro *et al.*, 2002). However, this impairment can be reversed with the administration of antioxidant vitamin C. This suggests that hyperinsulinemia increases oxidative stress in the vasculature (Arcaro *et al.*, 2002). Vascular oxidative stress and overproduction of ROS have a deleterious effect on eNOS activity and synthesis of NO. The ROS in insulin-resistant subjects enhance the oxidation of BH4 to 7,8-dihydrobiopterin (BH2), limiting the amount of active cofactor available for eNOS function. The activity of dihydropteridine reductase, an enzyme that regulates the rate of regeneration of BH4 from BH2, is reduced in insulin resistance and compounds on the problem with pteridine metabolism (Shinozaki *et al.*, 2001). Finally, ROS directly inactivate NO, thereby decreasing its bioavailability.

Nitric oxide (NO) demonstrates its important role in adipose tissue biology by influencing adipogenesis, insulin-stimulated glucose uptake, and lipolysis. Endothelial nitric oxide synthase (eNOS) is an enzyme responsible for NO formation in adipose cells (Engeli *et al.*, 2004). A study by Sansbury *et al.* (2012) demonstrate that increased eNOS activity prevents the obesogenic effects of high-fat diet without affecting systemic insulin resistance thereby stimulating metabolic activity in adipose tissue.

Ursolic acid (UA) is a natural pentacyclic triterpenoid carboxylic acid that is also known as urson, prunol, malol, or 3 β -hydroxyurs-12-en-28-oic acid (Mbaveng *et al.*, 2014). UA and its related compounds such as oleanolic acid, betulinic acid, uvaol or α - and β -amyrin, are widely distributed in various plants, and have become integral part of human diet (Jager *et al.*, 2009; Mbaveng *et al.*, 2014). Their content and composition differ between various species, due to the presence and activity of the enzymes responsible for their synthesis. Dietary sources of UA include apple (*Malus domestica*) fruit; apple peels contain large quantities of ursolic acid and related compounds (Cargnin & Gnoatto, 2017). Several studies, both in vitro and in vivo, have revealed that UA has diverse biological roles, including anti-inflammatory (Kashyap *et al.*, 2016), anti-oxidative (Liobikas *et al.*, 2011), anti-carcinogenic

(Shishodia *et al.*, 2003), anti-obesity (Jayaprakasam *et al.*, 2006), and anti-diabetic activities (Kwon *et al.*, 2018). We have shown that UA prevents the development of high-carbohydrate high-fat diet (HCHFD)-induced MS (Omodara *et al.*, 2022). However, the potential mechanism involved in the amelioration of vascular dysfunction and oxidative stress induced by HCHFD has not been explained. This study investigated the mechanism by which dietary UA supplementation improves vascular oxidative stress in male Wistar rats fed a HCHFD.

MATERIALS AND METHODS

Experimental Animals: A total of twenty (20) male Wistar rats, 8-9 weeks old and weighing 120 - 170 grams were housed in well-aerated plastic cages and allowed to acclimatize having free access to commercial grower mash feed and water *ad libitum*. After two weeks of acclimatization, the cages' beddings were changed from shaving sawdust to aluminium beddings. This allowed us to quantify spill over food and measure total daily food consumption.

Table 1:

Macronutrients in a normal diet and HCHF diet

Macronutrient	Normal diet	HCHF diet
Total carbohydrate (g/100g)	57.24	53.82
Total fat (g/100g)	4.78	17.29
Protein (g/100g)	25.76	14.05
Crude fibre (g/100g)	2.68	1.44
Ash (g/100g)	5.62	3.40
Moisture (g/100g)	4.06	10.02

Note:

Drinking water in the HCHFD-fed rats was augmented with 20% fructose.

Formulation of High-carbohydrate High-fat Diet (HCHFD):

The high-carbohydrate, high-fat diet (HCHFD) was formulated in-house following the method of Panchal *et al.* (2011) and Wong *et al.* (2017) with little modification. The high-carbohydrate, high-fat diet consists of condensed milk (39.5%), fructose (17.5%), thermally oxidized palm oil (20%), Powdered rat food (15.5%), Hubble, Mendel & Wakeman (HMW) salt mixture (2.5%), and water (5%) together with 20% fructose in drinking water (Omodara *et al.*, 2022). Proximate analysis of the diets was carried out by the method of AOAC (2006) and the results are presented in table 1 below.

Preparation of Thermally Oxidized Palm Oil: Fresh palm oil was thermally oxidized as described previously (Osim *et al.*, 1992). Briefly, fresh palm oil was subjected to heat at 150°C in a stainless-steel pot. The heating was for five rounds, and each round lasted 20 minutes. After each round, the oil was allowed to cool for 5 hours. The obtained thermally oxidized palm oil was then used in the formulation of the HCHFD.

Experimental Design: After two weeks of acclimatization, animals were randomly divided into 4 groups and treated as follows:

- (i) Group 1: Normal diet-fed rats + Distilled water (ND+DW; n = 5),
- (ii) Group 2: ND + Ursolic acid (ND+UA; n = 5),
- (iii) Group 3: High-carbohydrate high-fat diet-fed rats (HCHFD) + Distilled water (DW) (HCHFD + DW, n = 5),
- (iv) Group 4: HCHFD + Ursolic acid (HCHFD+UA, n = 5).

Feeding of Animals and Administration of Ursolic Acid:

The animals were fed their respective diets daily for 20 weeks. The ND-fed rats were provided with normal tap water while drinking water in the HCHFD-fed rats was augmented with 20% fructose. A dose of 250 mg/kg body weight of ursolic acid (Zhang *et al.*, 2016) was adopted. Ursolic acid was dissolved in an equal volume of distilled water and 50% DMSO, and administered orally through oral gavage to both ND+UA and HCHFD+UA groups starting 12 weeks after initiation of the HCHFD for a further 8 weeks period.

Determination of Percentage Change in Body Mass Index:

All rats were monitored daily for food and water intake. The body weight of rats was measured every 4 weeks from week 0 until week 12 and measured weekly from week 12 until week 20 using a standard weighing scale. While body length (nose to anus) and abdominal circumference were measured using a standard measuring tape every 4 weeks. The body mass index (BMI) of each rat was calculated as body weight (in grams) / [body length (in centimeters)]² (Panchal *et al.*, 2011). The difference between BMI values at weeks 0 and 20 were determined and used to calculate the percentage change (increase).

Calculation of Percentage Change or Increase:

Change = values at week 20 – values at week 0.

If the change is positive, it means there is an increase and if negative, it means there is a decrease. In this study, all changes were positive, meaning there were increases. Therefore, in this study, the percentage increase was calculated as follows:

Percentage increase (%) = increase/values at week 0 x 100.

Biochemical Analysis, Animal sacrifice, blood sample collection and tissue processing:

At the end of 20 weeks, animals were fasted overnight for 12 hours and anesthetized using a combination of 75 mg/kg body weight of ketamine and 5 mg/kg body weight of diazepam injection, and blood samples were collected via cardiac puncture (Flecknell, 2009). The blood samples, collected in plain bottles, were centrifuged for 10 minutes at 6000RPM to harvest the serum. The serum was collected, stored at -70°C and used for insulin and nitric oxide assay. Descending abdominal aorta and adipose tissues were isolated, washed in phosphate-buffered solution (pH 7.4) and homogenised with phosphate-buffered solution, centrifuged at 447 x g for approximately 20 min. The supernatant was carefully collected and used for the assay of reactive oxygen species, superoxide dismutase, catalase, glutathione and determination of expression levels of eNOS mRNA.

ELISA kits techniques: Insulin ELISA kits, nitric oxide (NO) ELISA kits, reactive oxygen species (ROS) ELISA Kits, Superoxide dismutase (SOD) ELISA kits, catalase

(CAT) ELISA kits and glutathione (GSH) ELISA kits were purchased from Coon Koon Biotech®, Shanghai, China. The assays were done following the manufacturer’s manual based on the principles of each of the test. ELISA microplate reader was used to read the absorbance and the concentration of the solutions.

Homeostasis Model Assessment for Insulin Resistance (HOMA-IR): Insulin resistance was determined by the formula:
HOMA-IR = fasting blood glucose (mg/dl) x fasting insulin (µiu/ml)/405 (Matthews *et al.*, 1985; Cho *et al.*, 2017).

Real-time polymerase chain reaction: The descending abdominal aorta and retroperitoneal adipose tissues were harvested and used to determine the levels of expression of eNOS mRNA by the real-time PCR method. Primer centrifuging was done using a micro refrigerated centrifuge (MTX-150) at 15,000 RPM. The primers were then reconstituted by using 150 µl of PCR graded water. Primer sequences were designed using the National Center for Biotechnology Information Primer Design tool. All primers were purchased from BIONEER, USA. The synthesized sequences of PCR primers are: eNOS – forward: 5'-TATTTGATGCTCGGGACTGC-3' and reverse: 5'-AAGATTGCCTCGGTTTGTG-3'. β-actin – forward: 5'-TTGTAACCAACTGGGACGATATGG-3' and reverse: 5'-GATCTTGATCTTGATGGTGCTGCTAGG-3'. β-actin primer was used as a housekeeping gene primer to amplify the endogenous control product (Padilla *et al.*, 2014). RNA extraction was carried out under ice rack. Samples were homogenized with a homogenizer and 500 µl of RB Buffer was added. The lysate was centrifuged at 13,000 RPM for 3 minutes and the supernatant was transferred into a new microcentrifuge tube. 200 µl of 100% ethanol was added and mix immediately using pipette. The sample was then transferred to a binding column in a 2 ml collection tube, the lid was closed and centrifuged at 14,000 RPM for 20 seconds. 700 µl of RWA1 was added without wetting the rim and centrifuged at 14,000 RPM for 20 seconds and supernatant was discarded. The sample was transferred into another collection tube and 500 µl of RWA2 Buffer was added without wetting the rim and centrifuged at 14,000 RPM for 20 seconds and supernatant also discarded. The sample was transferred into another collection tube and 500 µl of RWA2 Buffer was added without wetting the rim and centrifuged at 14,000 RPM for 4 minutes (2 minutes per orientation) and supernatant discarded. Ethanol was completely removed by centrifuging once more at 14,000 RPM for 1 minute. The binding column was then transferred to a new 1.5 ml tube for elution by adding 50 µl of ER Buffer and after waiting for 5 minutes at room temperature (25oC), we centrifuged at 10,000 RPM for 1 minute to elute. After total RNA extraction, cDNA was synthesized from total RNA using the High-Capacity cDNA Reverse Transcription kit (BIONEER, USA). 3 µl of each primer and 17 µl of each RNA, making 20 µl, were added to each reaction in the reverse transcription premix tube. The tubes were loaded in the PCR machine at 42oC for 1 hour. Quantitative real-time polymerase chain reaction (PCR) was performed using the Real-Time PCR Detection System (Applied Biosystem GeneAmpR). Primers and cDNA were thawed. A 20-µL reaction mixture containing 10-µL of real-

time PCR reagent (AccuPowerR 2X GreenStar™ qPCR Master Mix, BIONEER, USA), 4-µL of gene-specific primers (2-µL for forward primer and 2-µL for reverse primer) plus 4-µL of cDNA template, together with appropriate concentration of PCR graded water and ROX dye in PCR tubes. The mixture was mixed by vortexing and the tubes were sealed using optical adhesive film for real-time PCR. Centrifuging was done at 3,000 RPM for 2 minutes and the tubes were loaded into the Real-Time PCR Detection System. The reactions were performed under the following conditions:

Table 2:

PCR conditions			
Step	Temperature	Time	Cycles
Pre-denaturation	94 °C	5 min	1 cycle
Denaturation	94 °C	30 sec	40
Annealing	54 °C	30 sec	Cycles
Extension	72 °C	30 sec	
Final extension	72 °C	5 min	

Thereafter, mRNA expression level was determined as fold change (FC) as follows:

$$FC = 2^{(-\Delta\Delta Ct)}$$
 (Livak and Schmittgen, 2001).

Statistical Analysis: Data were expressed as mean ± standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. Values at P<0.05 was considered statistically significant. The SPSS version 23 (IBM, Armonk, NY, USA) was used for statistical analysis.

RESULTS

Effect of ursolic acid on body mass index in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 1 shows that the HCHFD+DW group had a significantly (P<0.05) higher BMI increase (50.0±0.69) compared to the ND+DW group (23.84±0.57). While the HCHFD+UA-fed rats had a significantly (P<0.05) lower BMI increase (34.78±0.30) compared to the HCHFD+DW group (50.0±0.69).

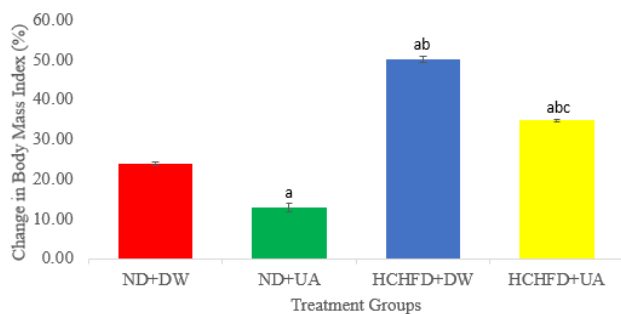


Figure 1: Percentage change in body mass index in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at P<0.05; b = significant vs ND+UA at P<0.05; c = significant vs HCHFD+DW at P<0.05. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on fasting serum insulin in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 2 shows that there was a significant increase ($P<0.05$) in fasting insulin level in the HCHFD+DW group (123.76 ± 0.95) compared to the ND+DW group (73.84 ± 0.72). The HCHFD+UA-fed animals had a significantly lower ($P<0.05$) insulin level (95.64 ± 0.48) compared to the HCHFD+DW group (123.76 ± 0.95).

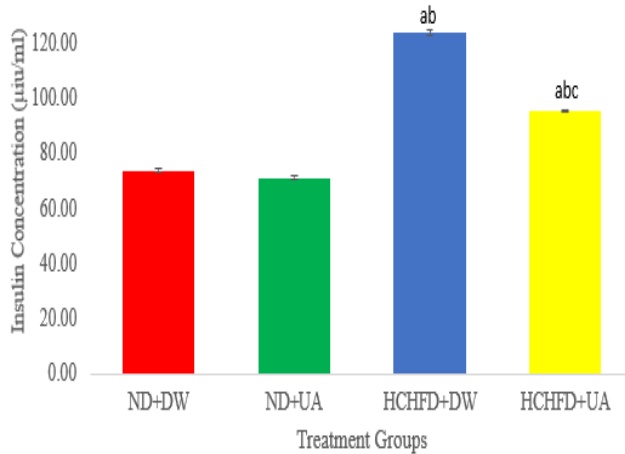


Figure 2:

Serum insulin concentration in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group

Effect of ursolic acid on homeostatic model assessment for insulin resistance in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 3 shows that there was a significant increase ($P<0.05$) in HOMA-IR level in the HCHFD+DW group (36.72 ± 0.53) compared to the ND+DW group (16.70 ± 0.34). The HCHFD+UA-fed animals had a significantly lower ($P<0.05$) HOMA-IR level (23.0 ± 0.34) compared to the HCHFD+DW group (36.72 ± 0.53).

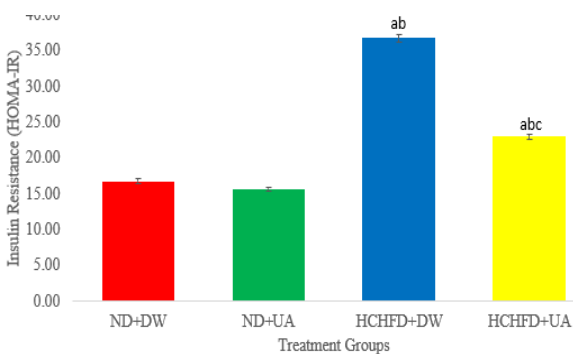


Figure 3:

Homeostatic model assessment for insulin resistance in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on aorta superoxide dismutase in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 4 shows that there was a significant decrease ($P<0.05$) in the level of aorta SOD in the HCHFD+DW group (28.82 ± 1.02) compared to the ND+DW group (48.68 ± 1.10). The HCHFD+UA-fed animals had a significantly higher ($P<0.05$) level of aorta SOD (49.34 ± 1.14) compared to the HCHFD+DW group (28.82 ± 1.02).

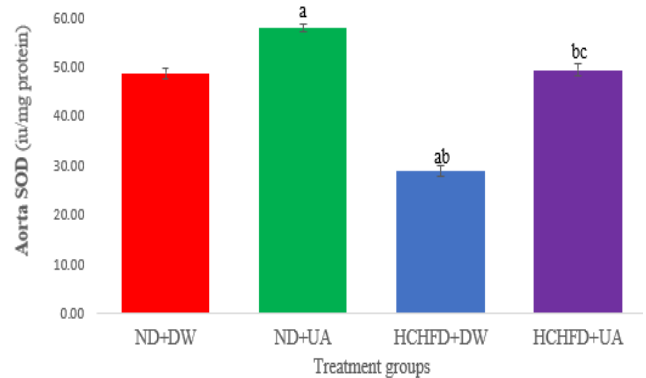


Figure 4:

Levels of aorta superoxide dismutase in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on aorta catalase in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 5 shows that there was a significant decrease ($P<0.05$) in the level of aorta catalase in the HCHFD+DW group (39.50 ± 1.50) compared to the ND+DW group (53.32 ± 2.09). The HCHFD+UA-fed animals had a significantly higher ($P<0.05$) level of aorta catalase (57.84 ± 1.50) compared to the HCHFD+DW group (39.50 ± 1.50).

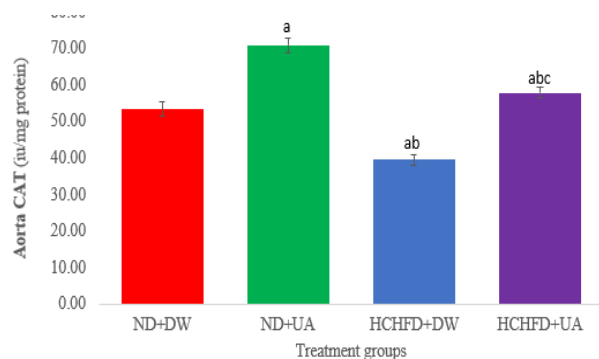


Figure 5:

Levels of aorta catalase in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on aorta glutathione in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 6 shows that there was a significant decrease ($P<0.05$) in the level of aorta glutathione in the HCHFD+DW group (9.76 ± 0.19) compared to the ND+DW group (14.58 ± 0.41). The HCHFD+UA-fed animals had a significantly higher ($P<0.05$) level of aorta glutathione (13.58 ± 0.42) compared to the HCHFD+DW group (9.76 ± 0.19).

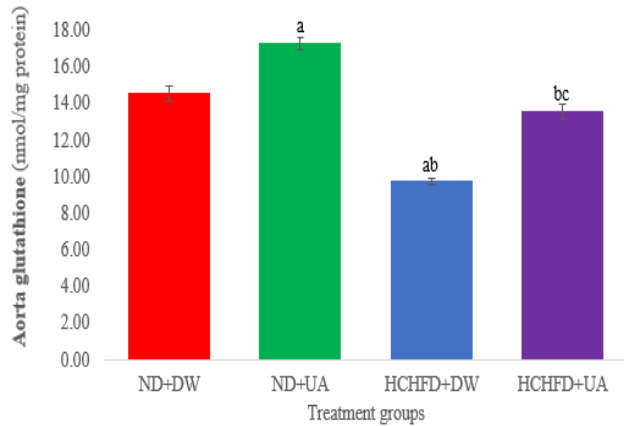


Figure 6:

Levels of aorta glutathione in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on serum nitric oxide in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 7 shows that level of serum NO significantly decreased ($P<0.05$) in the HCHFD+DW group (24.30 ± 1.01) compared to the ND+DW group (28.50 ± 0.96). There was a significant increase ($P<0.05$) in the level of serum NO in the HCHFD+UA-fed animals (38.96 ± 0.97) compared to the HCHFD+DW group (24.30 ± 1.01).

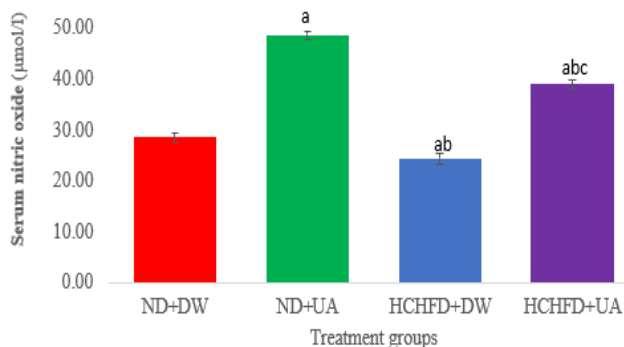


Figure 7:

Levels of serum nitric oxide in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on aorta reactive oxygen species in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 8 shows that level of aorta ROS significantly increased ($P<0.05$) in the HCHFD+DW group (521.76 ± 0.84) compared to the ND+DW group (259.02 ± 0.44). There was a significant decrease ($P<0.05$) in the level of aorta ROS in the HCHFD+UA-fed animals (331.00 ± 0.52) compared to the HCHFD+DW group (521.76 ± 0.84).

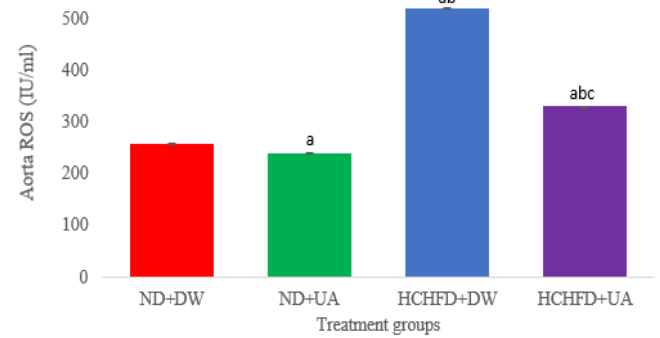


Figure 8:

Levels of serum nitric oxide in male Wistar rats fed HCHFD and UA. (n = 5 rats per group). UA dose = 250 mg/kg body weight. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on aorta eNOS mRNA expression in male Wistar rats fed a high-carbohydrate high-fat diet. Figure 9 shows that aortic eNOS mRNA level was significantly ($P<0.05$) downregulated in the HCHFD+DW-fed rats (0.26 ± 0.06) compared to normal control (1.00 ± 0.04). Treatment with UA significantly ($P<0.05$) upregulated aortic eNOS mRNA level in the HCHFD+UA-fed rats (0.92 ± 0.03) compared to the HCHFD+DW-fed rats (0.26 ± 0.06).

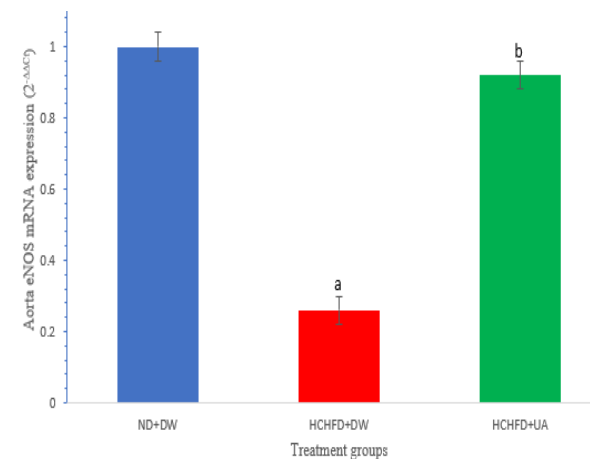
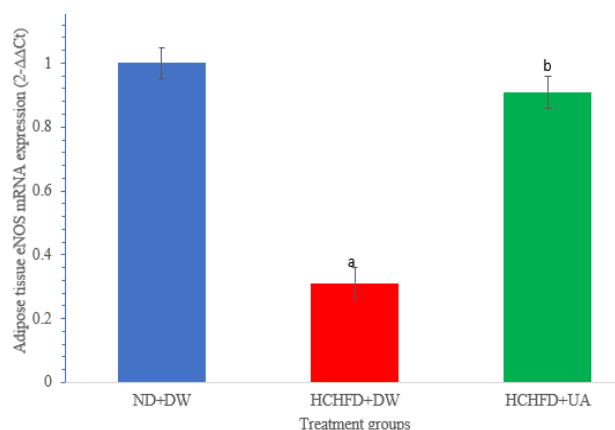


Figure 9:

Aortic eNOS mRNA expression in HCHFD and ursolic acid-treated male Wistar rats. (n = 5 rats per group). UA dose = 250 mg/kg body weight. ND+DW: normal diet + distilled water group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group. a = significant vs ND+DW; b = significant vs HCHFD+DW, at $P<0.05$.

**Figure 10:**

Adipose tissue eNOS mRNA expression in HCHFD and ursolic acid-treated male Wistar rats. (n = 5 rats per group). UA dose = 250 mg/kg body.

ND+DW: normal diet + distilled water group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

a = significant vs ND+DW; b = significant vs HCHFD+DW, at $P < 0.05$

Effect of ursolic acid on adipose tissue eNOS mRNA expression in male Wistar rats fed a high-carbohydrate high-fat diet: Figure 10 shows that adipose tissue eNOS mRNA level was significantly ($P < 0.05$) downregulated in the HCHFD+DW-fed rats (0.31 ± 0.04) compared to normal control (1.00 ± 0.05). Treatment with UA significantly ($P < 0.05$) upregulated adipose tissue eNOS mRNA level in the HCHFD+UA-fed rats (0.91 ± 0.06) compared to HCHFD+DW-fed rats (0.31 ± 0.04).

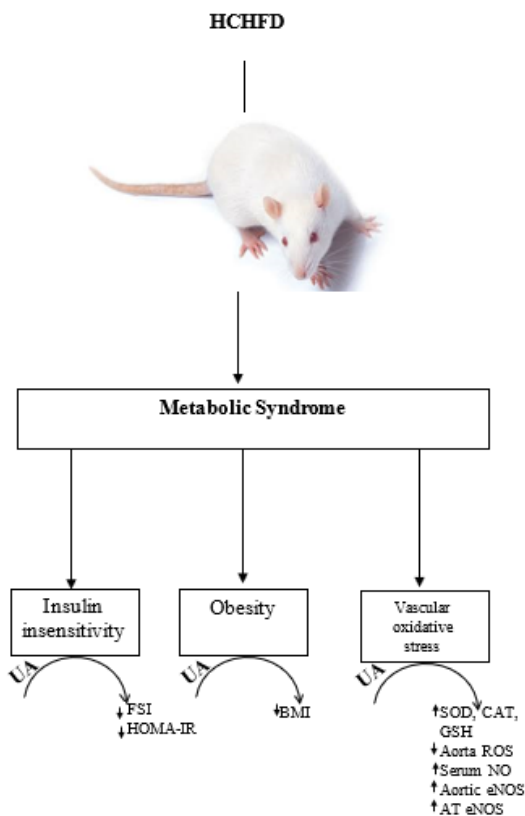
DISCUSSION

The results of this study did not only demonstrate metabolic syndrome-related HCHFD-induced vascular oxidative stress, but also showed that ursolic acid has the potential to ameliorate this effect.

The results of this study showed that HCHFD increased body mass index (BMI). BMI is a marker of obesity (Novelli *et al.*, 2007). According to Lobato *et al.* (2012), the severity of vascular endothelial dysfunction strongly correlates with the degree of visceral adiposity, probably due to adipocyte hypertrophy. Also, 20 weeks of feeding on HCHFD increased serum insulin and HOMA-IR. The increased HOMA-IR, which is as a result of increased fasting blood glucose and serum insulin, suggests insulin resistance. High fat feeding in mice has been shown to induce insulin resistance. (Zabolotny *et al.*, 2008; Agouni *et al.*, 2011). Insulin resistance has been linked to vascular dysfunction (Eringa *et al.*, 2007).

The findings from this study showed that HCHFD significantly decreased aorta antioxidant enzymes (SOD, CAT and GSH). This finding suggests increased vascular oxidative stress (Panchal *et al.*, 2011). This study also showed that HCHFD significantly increased levels of aorta reactive oxygen species (ROS). Fructose feeding is associated with the induction of oxidative stress (Stanhope & Havel, 2008). An imbalance between the production of ROS and the ability of the antioxidant systems to readily detoxify these reactive intermediates results in oxidative

stress (Stanhope & Havel, 2008). Significant increase in aortic free radicals combined with decreased aortic antioxidant defense systems is an indication of vascular oxidative stress. Free radicals generated in excessive and uncontrollable amounts under oxidative stress conditions cause damage to DNA, proteins, and lipids, which can severely compromise cell health and contribute to disease development (Birben *et al.*, 2012).



↓ = Decrease, ↑ = Increase. FSI – fasting serum insulin, HOMA-IR – homeostatic assessment for insulin resistance, BMI – body mass index, SOD – superoxide dismutase, CAT – catalase, GSH – glutathione, ROS – reactive oxygen species, NO – nitric oxide, AT – adipose tissue, eNOS – endothelial nitric oxide synthase, UA – ursolic acid, HCHFD – high-carbohydrate high-fat diet

Figure 11:

Proposed mechanism by which ursolic acid ameliorate metabolic syndrome-related HCHFD-induced vascular dysfunction

Results from this study also showed that HCHFD significantly decreased serum nitric oxide (NO) levels, as well as aortic and adipose tissue eNOS mRNA levels. This corroborate the findings of previous studies where they reported that serum NO was reduced (Panchal *et al.*, 2011) and eNOS was downregulated (Sansbury and Hill, 2015) in obese rats. Reduced bioavailability of NO is commonly attributed to downregulation of eNOS (Sansbury and Hill, 2015). Inflammatory state occurring in metabolic syndrome has been linked to vascular dysfunction. The cytokine, TNF- α , which is implicated in the initiation of insulin resistance, downregulates eNOS abundance by decreasing the stability of eNOS mRNA (Sansbury and Hill, 2015). Vascular endothelial dysfunction is not only a consequence of insulin

resistance, but also impairs insulin signaling to further reduce insulin sensitivity, thereby resulting in a destructive cycle in metabolic syndrome and diabetes. In obese rats, altered insulin signaling disrupts insulin-mediated NO production via downregulation of eNOS expression to impair vasodilatation in resistance arteries. The involvement of ROS and subsequent degradation of BH4 (a cofactor essential for NO synthesis from eNOS) in insulin resistance is thought to play a role in the impairment of NO-dependent vasodilatation (Eringa *et al.*, 2007; Trans *et al.*, 2020).

As shown in figure 11, in this present study, obesity, fasting hyperinsulinemia, insulin resistance and vascular oxidative stress, all induced by a 20-weeks feeding on HCHFD were significantly alleviated by UA. Zhang *et al.* (2016) and Ramirez-Rodriguez *et al.* (2017) have reported that the anti-obesity effect of UA occurs possibly via increased Akt phosphorylation and improved skeletal muscle glucose uptake. In this study, the decreased serum insulin and amelioration of insulin resistance by UA suggests that UA may have an insulin sensitizing effect.

The findings of this study also showed that UA significantly increased vascular antioxidant enzymes. This corroborate the findings of Mason *et al.* (2020) and may suggest an enhancement of the antioxidant defense system. In this present study, UA significantly decreased aortic ROS level. This is consistent with the findings of Saad *et al.* (2015) and Pordanjani *et al.* (2022).

Findings from this study showed that UA mitigates vascular oxidative stress through its antioxidative actions demonstrated by decreased aortic free radicals which were probably scavenged by the enhanced antioxidant defense systems.

Also, UA may have the potential to enhance endothelium-derived vasorelaxation (EDVR) due to its ability to significantly increase serum NO levels, as well as aortic and AT eNOS mRNA. The restoration of insulin sensitizing effect by UA as earlier observed, may have synergistic effect with NO to increase the vascular antioxidant defense systems and consequently improve EDVR.

In conclusion, findings from this study demonstrated that 20 weeks of feeding on HCHFD successfully induced metabolic syndrome with features such as obesity, insulin resistance, oxidative stress and impaired vasorelaxation in aortic vasculature of male Wistar rats. However, 8 weeks supplementation with UA ameliorated metabolic syndrome-related insulin insensitivity and oxidative stress in aortic vasculature of HCHFD-fed male Wistar rats and upregulates both aortic and adipose tissue eNOS mRNA. Therefore, it was concluded that UA ameliorates vascular oxidative stress and upregulates eNOS gene in male Wistar rats with HCHFD-induced metabolic syndrome.

Further studies, including vascular reactivity and protein expression studies in aortic vasculature, are recommended.

REFERENCES

- Agouni, A. M., Ody, N., Owen, C. (2011). Liver-specific deletion of protein tyrosine phosphatase (PTP) 1B improves obesity- and pharmacologically-induced endoplasmic reticulum stress. *Biochemistry Journal*, 438 (2), 369-378.
- AOAC (Association of Official Analytical Chemists), (2006). Official Method of Analysis of the AOAC (W. Horwitz Editor) Eighteenth Edition. Washington D.C, AOAC.
- Arcaro, G., Cretti, A., Balzano, S., Lechi, A., Muggeo, M., Bonora, E., Bonadonna, R. C. (2002). Insulin causes endothelial dysfunction in humans: sites and mechanisms. *Circulation*, 105(5), 576-582.
- Ashfaq, S., Abramson, J. L., Jones, D. P., Rhodes, S. D., Weintraub, W. S., Hooper, W. C., Alexander, R. W., Vaccarino, V., Harrison, D. G., Quyyumi, A. A. (2008). Endothelial function and aminothiol biomarkers of oxidative stress in healthy adults. *Hypertension*, 52(1), 80-85.
- Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., Kalayci, O. (2012). "Oxidative stress and antioxidant defense," *The World Allergy Organization Journal*, 5 (1), 9-19.
- Bohlen, H. G., Zhou, X., Unthank, J. L., Miller, S. J., Bills, R. (2009). Transfer of nitric oxide by blood from upstream to downstream resistance vessels causes microvascular dilation. *American Journal of Physiology- Heart and Circulatory Physiology*, 297(4), H1337-H1346.
- Cargnin, S. T., Gnoatto, S. B. (2017). "Ursolic acid from apple pomace and traditional plants: A valuable triterpenoid with functional properties". *Food Chemistry*, 220, 477-489.
- Cho, J., Hong, H., Park, S., Kim, S., Kang, H. (2017). Insulin resistance and its association with metabolic syndrome in Korean Children. *Biomedical Research International* 2017: 8728017.
- Corson, M. A., James, N. L., Latta, S. E., Nerem, R. M., Berk, B. C., Harrison, D. G. (1996). Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. *Circulation Research*, 79(5), 984-991.
- De Ciuceis, C., Amiri, F., Brassard, P., Endemann, D. H., Touyz, R. M., Schiffrin, E. L. (2005). Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II-infused macrophage colony-stimulating factor-deficient mice: evidence for a role in inflammation in angiotensin-induced vascular injury. *Arteriosclerosis, Thrombosis and Vascular Biology*, 25, 2106-2113.
- Dhawan, S. S., Eshtehardi, P., McDaniel, M. C., Fike, L. V., Jones, D. P., Quyyumi, A. A., Samady, H. (2011). The role of plasma aminothiols in the prediction of coronary microvascular dysfunction and plaque vulnerability. *Atherosclerosis*, 219(1), 266-272.
- Di, W. H., Hope, S., Du, Y., Quinn, M. T., Cayatte, A., Pagano, P. J., Cohen, R. A. (1999). Paracrine role of adventitial superoxide anion in mediating spontaneous tone of the isolated rat aorta in angiotensin II-induced hypertension. *Hypertension*, 33, 1225-1232.
- Engeli, S., Janke, J., Gorzelnjak, K., Bohnke, J., Ghose, N., Lindschau, C., Luft, F. C., Sharma, A. M. (2004). Regulation of the nitric oxide system in human adipose tissue. *Journal of Lipid Research*, 45 (9): 1640-1648.
- Eringa, E. C., Stehouwer, C. D., Roos, M. H., Westerhof, N., Sipkema, P. (2007). Selective resistance to vasoactive effects of insulin in muscle resistance arteries of obese Zucker (fa/fa) rats. *Am. J. Physiol. Endocrinol. Metab.* 293 (5), 1134-1139. 10.1152/ajpendo.00516.2006.
- Flecknell, P. (2009). Laboratory animal anaesthesia. Elsevier Inc; 3rd edition, 181-190.
- Forstermann, U., Sessa, W. C. (2012). Nitric oxide synthases: regulation and function. *European Heart Journal*, 33(7), 829-837, 837a-837d.
- Förstermann, U., Mülsch, A., Böhme, E., Busse, R. (1986). Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived factor from

- rabbit and canine arteries. *Circulation Research*, 58(4), 531–538.
- Greenstein, A. S., Khavandi, K., Withers, S. B., Sonoyama, K., Clancy, O., Jeziorska, M., Laing, I., Yates, A. P., Pemberton, P. W., Malik, R. A., Heagerty, A. M. (2009). Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. *Circulation*, 119, 1661–1670.
- Jager, S., Trojan, H., Kopp, T., Laszczyk, M. N., Scheffler, A. (2009). Pentacyclic triterpene distribution in various plants - rich sources for a new group of multi-potent plant extracts. *Molecules*, 14, 2016–2031.
- Jayaprakasam, B., Olson, L. K., Schutzki, R. E., Tai, M. H., Nair, M. G. (2006). Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). *Journal of Agricultural and Food Chemistry*, 54: 243–248.
- Johnstone, M. T., Creager, S. J., Scales, K. M., Cusco, J. A., Lee, B. K., Creager, M. A. (1993). Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation*, 88(6), 2510–2516.
- Kashyap, D., Sharma, A., Tuli, H. S., Punia, S., Sharma, A. K. (2016). Ursolic acid and oleanolic acid: pentacyclic terpenoids with promising antiinflammatory activities. *Recent Patents on Inflammation & Allergy Drug Discovery*, 10: 21–33.
- Klatt, P., Pfeiffer, S., List, B. M. (1996). Characterization of heme-deficient neuronal nitric-oxide synthase reveals a role for heme in subunit dimerization and binding of the amino acid substrate and tetrahydrobiopterin. *Journal of Biological Chemistry*, 271(13), 7336–7342.
- Kwon, E. Y., Shin, S. K., Choi, M. S. (2018). Ursolic Acid Attenuates Hepatic Steatosis, Fibrosis, and Insulin Resistance by Modulating the Circadian Rhythm Pathway in Diet-Induced Obese Mice. *Nutrients*, 10: 1-15.
- Lam, C. F., Peterson, T. E., Richardson, D. M. (2006). Increased blood flow causes coordinated upregulation of arterial eNOS and biosynthesis of tetrahydrobiopterin. *American Journal of Physiology – Heart and Circulatory Physiology*, 290(2), H786–H793.
- Lee, S. K., Khambhati, J., Bhargava, A., Engels, M. C., Sandesara, P. B., Quyyumi, A. A. (2017). Endothelial Dysfunction and Metabolic Syndrome. *Hypertension Journal*, 3(2), 72-80.
- Liobikas, J., Majiene, D., Trumbeckaite, S., Kursvietiene, L., Masteikova, R., Kopustinskiene, D. M., Savickas, A., Bernatoniene, J. (2011). Uncoupling and antioxidant effects of ursolic acid in isolated rat heart mitochondria. *Journal of Natural Product*, 74: 1640–1644.
- Livak, K. J., Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods San Diego Calif* 25:402–8.
- Mason, S. A., Trewin, A. J., Parker, L., Wadley, G. D. (2020). Antioxidant supplements and endurance exercise: Current evidence and mechanistic insights. *Redox Biology*; 35: 101471.
- Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–19.
- Mbaveng, A. T., Hamm, R., Kuete, V. (2014). Harmful and Protective Effects of Terpenoids from African Medicinal Plants. *Toxicological Survey of African Medicinal Plants, Elsevier Inc*; 557-576, ISBN: 9780128000182.
- Meyers, M. R., Gokce, N. (2007). Endothelial dysfunction in obesity: etiological role in atherosclerosis. *Current opinion of endocrinology, diabetes and obesity*. 14(5), 365-369.
- Mkhwanazi, B. N., Serumula, M. R., Myburg, R. B., Van Heerden, F. R., Musabayane, C. T. (2014). Antioxidant effect of maslinic acid in livers, hearts and kidneys of streptozotocin-induced diabetic rats: effects on kidney function. *Renal failure*, 36(3), 419-431.
- Molyneux, C. A., Glyn, M. C., Ward, B. J. (2002). Oxidative stress and cardiac microvascular structure in ischemia and reperfusion: the protective effect of antioxidant vitamins. *Microvascular research*, 64(2), 265-277.
- Novelli, E. L., Diniz, Y. S., Galhardi, C. M., Ebaid, G. M., Rodrigues, H. G., Mani, F., Fernandes, A. A., Cicogna, A. C., Novelli Filho, J. L. (2007). Anthropometrical parameters and markers of obesity in rats. *Laboratory Animals* 41(1): 111-9.
- O'Neill, S., O'Driscoll, L. (2015). Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes Rev.*; 16 (1), 1–12. 10.1111/obr.12229.
- Omodara, O. O., Kawu, M. U., Bako, I. G. (2022). Ursolic acid prevents the development of metabolic syndrome in male Wistar rats fed a high-carbohydrate high-fat diet. *Journal of African Association of Physiological Sciences*, 10(1): 1-12.
- Osim, E. E., Owu, D. U., Isong, E. U., Umoh, I. B. (1992). Influence of chronic consumption of thermoxidized palm oil diet on platelet aggregation in rat. *Discovery and Innovation*, 4: 83087.
- Padilla, J., Jenkins, N. T., Thorne, P. K., Lansford, K. A., Fleming, N. J., Bayless, D. S., Sheldon, R. D., Rector, R. S., Laughlin, M. H. (2014). Differential regulation of adipose tissue and vascular inflammatory gene expression by chronic systemic inhibition of NOS in lean and obese rats. *Physiological Reports* 2(2): 1-16.
- Panchal, S. K., Poudyal, H., Iyer, A., Nazer, R., Alam, M., Diwan, V., Kauter, K., Sernia, C., Campbell, F., Ward, L., Gobe, G., Fenning, A., Brown, L. (2011). High-carbohydrate, High-fat Diet-induced Metabolic Syndrome and Cardiovascular Remodeling in Rats. *Journal of Cardiovascular Pharmacology*, 57: 611–624.
- Patel, R. S., Al Mheid, I., Morris, A. A., Ahmed, Y., Kavtaradze, N., Ali, S., Uphoff, I., Sher, S., Dabhadkar, K., Aznaouridis, K. (2011). The oxidized aminothiols cysteine is associated with impaired arterial indices in healthy human subjects. *Atherosclerosis*, 218(1), 90-95.
- Pordanjani, M. K., Banitalebi, E., Roghani, M., Hemmati, R. (2022). Ursolic acid enhances training on vascular aging by reducing oxidative stress in aged type 2 diabetic rats. *Food Sciences & Nutrition*; 11(2): 696-708; <https://doi.org/10.1002/fsn.3.3105>.
- Ramírez-Rodríguez, A. M., González-Ortiz, M., Martínez-Abundis, E., Acuña Ortega, N. (2017). Effect of ursolic acid on metabolic syndrome, insulin sensitivity, and inflammation. *Journal of Medicinal Food* 20: 882–886.
- Rapoport, R. M., Draznin, M. B., Murad, F. (1983). Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature*, 306(5949), 174–176.
- Saad, E. A., Hassanien, M. M., El-Hagrasy, M. A., Radwan, K. H. (2015). Antidiabetic, hypolipidemic and antioxidant activities and protective effects of Punica granatum peels powder against pancreatic and hepatic tissues injuries in streptozotocin-induced IDDM in rats. *International Journal of Pharmacy and Pharmaceutical Sciences*; 7(7): 397-402.
- Sansbury, B. E., Cummins, T. D., Tang, Y., Hellmann, J., Holden, C. R., Harbeson, M. A., Chen, Y., Patel, R. P., Spite, M., Bhatnagar, A., Hill, B. G. (2012). Overexpression of

- endothelial nitric oxide synthase prevents diet-induced obesity and regulates adipocyte phenotype. *Circulation Research*, 111: 1176-1189.
- Sansbury, B. E., Hill, B. G. (2015). Anti-obesogenic role of endothelial nitric oxide synthase. *Vitamins and Hormones*, 96, 323-346.
- Schiffrin, E. L., Touyz, R. M. (2004). From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension. *American Journal of Physiology – Heart and Circulatory Physiology*, 287, H435–H446.
- Schiffrin, E. L. (2008). Oxidative stress, nitric oxide synthase, and superoxide dismutase: a matter of imbalance underlies endothelial dysfunction in the human coronary circulation. *Hypertension*, 51, 31–32.
- Schillaci, G., Pirro, M., Vaudo, G., Mannarino, M. R., Savarese, G., Pucci, G., Franklin, S. S., Mannarino, E. (2005). Metabolic syndrome is associated with aortic stiffness in untreated essential hypertension. *Hypertension*, 45, 1078–1082.
- Shaul, P. W. (2002). Regulation of endothelial nitric oxide synthase: location, location, location. *Annual Review of Physiology*, 64, 749–774.
- Shinozaki, K., Hirayama, A., Nishio, Y., Yoshida, Y., Ohtani, T., Okamura, T., Masada, M., Kikkawa, R., Kodama, K., Kashiwagi, A. (2001). Coronary endothelial dysfunction in the insulin-resistant state is linked to abnormal pteridine metabolism and vascular oxidative stress. *Journal of American College of Cardiology*, 38(7), 1821-1828.
- Shishodia, S., Majumdar, S., Banerjee, S., Aggarwal, B. B. (2003). Ursolic acid inhibits nuclear factor-kappaB activation induced by carcinogenic agents through suppression of IkappaBalpha kinase and p65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. *Cancer Research*, 63: 4375–4383.
- Stanhope, K. L., Havel, P. J. (2008). Fructose consumption: potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance. *Current Opinion in Lipidology*, 19, 16–24.
- Tran, V., De Silva, T. M., Sobey, C. G., Lim, K., Drummond, G. R., Vinh, A., Jelinic, M. (2020). The vascular consequences of metabolic syndrome: Rodent models, endothelial dysfunction, and current therapy. *Front Pharmacol.*; 11: 148. doi: 10.3389/fphar.2020.00148.
- Tune, J. D., Goodwill, A. G., Sassoon, D. J., Mather, K. J. (2017). Cardiovascular consequences of metabolic syndrome. *Transl. Res.*; 183, 57–70. 10.1016/j.trsl.2017.01.001.
- Wong, S. K., Chin, K. Y., Suhaimi, F. H., Ahmad, F., Ima-Nirwana, S. (2017) The Effects of a Modified High-carbohydrate High-fat Diet on Metabolic Syndrome Parameters in Male Rats. *Experimental and Clinical Endocrinology and Diabetes*, DOI <https://doi.org/10.1055/s-0043-119352>.
- World Health Organization (2022). World Obesity Day 2022 – Accelerating action to stop obesity. Available online: <https://www.who.int/news/item/04-03-2022-world-obesity-day-2022-accelerating-action-to-stop-obesity> (accessed on 8 August 2022).
- Zabolotny, J. M., Kim, Y. B., Welsh, L. A., Kershaw, E. E., Neel, B. G., Khan, B. B. (2008). Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. *Journal of Biological Chemistry*, 283 (21), 14230–14241.
- Zhang, Y., Song, C., Li, H., Hou, J., Li, D. (2016). Ursolic acid prevents augmented peripheral inflammation and inflammatory hyperalgesia in high-fat diet-induced obese rats by restoring downregulated spinal PPARα. *Molecular Medicine Reports*, 13: 5309-5316.
- Zhang, Y., Song, C., Li, H., Hou, J., Li, D. (2016). Ursolic acid prevents augmented peripheral inflammation and inflammatory hyperalgesia in high-fat diet-induced obese rats by restoring downregulated spinal PPARα. *Molecular Medicine Reports*, 13: 5309-5316..

Full length Research Article

Changes in the Subcortical White Matter and the Pyramidal Neurons in the Sensorimotor Cortex of Juvenile Hydrocephalic Rats

Femi Akinlosotu O.M., *Olopade F.E., Okoye C.S. and Shokunbi M.T.

Developmental Neurobiology and Forensic Anatomy Unit, Department of Anatomy, College of Medicine,
University of Ibadan, Ibadan, Nigeria

Summary: Hydrocephalus, the aberrant enlargement of the brain's ventricles, is caused by a build-up of cerebrospinal fluid that stretches the periventricular white matter and may disrupt the connections between the pyramidal neurons in the cerebral cortex. Using an intra-cisternal kaolin injection hydrocephalic rat model, we aimed to determine if the effects of hydrocephalus on the white matter will subsequently impact the dendrites of cortical pyramidal neurons, which are the synaptic sites for the white matter fibres, particularly the afferent fibres. Three-week-old hydrocephalic rats were compared with controls after 1 week, 2 weeks and 4 weeks. Dendritic arborisations of the pyramidal neurons were evaluated using a modified Golgi stain. Haematoxylin and Eosin, and Cresyl violet stains were used to measure cortical thickness and pyramidal neuronal count. The hydrocephalic rats' cerebral cortex and corpus callosum were thinner ($p < 0.0001$) than the controls. Rats with hydrocephalus had a similar pyramidal neuron count to the controls, with no significant decline. After two weeks of hydrocephalic induction, the pyramidal neurons' dendritic branching changed due to basal dendritic reduction and denudation. Reduced thickness in the corpus callosum and sensorimotor cortex was observed in the hydrocephalus animals. The pyramidal cell population remained unaffected, but the basal dendrites of the cells were substantially weakened. Ventricular enlargements during the juvenile developmental stage may harm dendritic arborization, which could obstruct neurological maturation.

Keywords: hydrocephalus, Golgi stain, pyramidal neurons, cortical thickness, corpus callosum, dendritic architecture.

*Authors for correspondence: funmiolopade@yahoo.com, Tel: +234- 8052274102

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INTRODUCTION

Hydrocephalus is a neurological disorder classically considered a failure of cerebrospinal fluid (CSF) homeostasis from its point of production within the ventricular system to its point of absorption into the systemic circulation resulting in the active distension of the cerebral ventricles. This damage occurs in a multifactorial way, particularly as ventriculomegaly becomes more severe, with the prevalence of cortical thinning and distention, along with stretching of the septum pellucidum (Maller & Gray, 2016; Rekate & Blitz, 2016; Kahle *et al.*, 2016, 2024). Neocortical pyramidal neurons are cerebral cortex's fundamental building blocks and the most prevalent type, with their soma located in layers V and VI. While the apical dendrites ascend towards the pia, basal dendrites fan out around the soma. During this process, they often give off oblique dendrites and end in a tuft of dendrites in layer 1 or other layers. Except for sporadic aberrant spines, both basal and apical dendrites normally have a high density of spines. A significant portion of the neocortex's output comes from these pyramidal neurons. Input signals from numerous brain regions' afferent fibers are combined, processed through

several layers, and then transmitted to various locations via these pyramidal neurons. Excitatory synapses in the cortex are primarily derived from these cells, and the primary postsynaptic targets of excitatory synapses are their dendritic spines, or simply spines (Benavides-Piccione *et al.*, 2021; Nolan *et al.*, 2024). Their dendritic structures differ significantly between cortical areas, layers, and species. How they integrate information is greatly influenced by these variations in their neuronal architecture. They also contribute to almost all intra-areal, inter-areal, and subcortical projections, and they are the principal recipients of excitatory synapses (Chen *et al.*, 2017; Galakhova *et al.*, 2022; Hunt *et al.*, 2023; Benavides-Piccione *et al.*, 2024). Because of their greater size and complexity, dendritic trees offer greater computational diversity. Dendritic branches can function as distinct processing units on their own and perform discrete computations (Galakhova *et al.*, 2022; Benavides-Piccione *et al.*, 2024). Utilizing an intra-cisternal kaolin injection hydrocephalic rat model three weeks post-delivery and weaning, the present study is unique in that it measures the dendritic arborizations, cortical thickness and neuronal count of the pyramidal neurons in the cerebral

cortex. This study focuses on the pyramidal neurons of layer V of the cerebral cortex in young hydrocephalic rats to provide insight into the fate of their dendrites. We postulate that the impact of hydrocephalus on the white matter, which is composed of both afferent and efferent fibers, will subsequently influence the cerebral cortex's pyramidal neurons' dendrites, which serve as the sites of synapses for the white matter fibers, particularly the afferent fibers (Del Bigio, 2010; Chen *et al.*, 2017; Curzio & Curzio, 2017).

MATERIALS AND METHODS

Animal Procurement, Care, and Management: All procedures on animal handling were conducted in compliance with the protocol of Institutional Animal Care and Use Committee of the National Institute of Health, USA, European Committee Council Directives (86/609/EEC) and ARRIVE guidelines, minimizing the number of animals used and avoiding their suffering. Fifteen sexually matured female and five male Wistar rats were procured from the Central Animal House of the Faculty of Basic Medical Sciences, University of Ibadan. They were mated in a 3:1 ratio of females to males per cage and housed in the Animal House of the Faculty of Basic Medical Science under normal temperature ($32 \pm 2^\circ\text{C}$) and natural light/dark cycle conditions with ad libitum access to standard rat chow and water. Pregnant rats were housed separately until delivery and with their pups until the weaning period (postnatal day 21). At weaning, the pups were separated from the dams and hydrocephalus was induced.

Induction of hydrocephalus: Hydrocephalus was induced in the 3-week-old rats (26–28g) under ketamine/xylazine anaesthesia at 90/10mg/kg i.p. The cervical region of the rats was dapped with an ethanol-moistened cotton pad and flexed. The sub-occipital region for the rhomboid fossa marking the cisterna magna was detected by palpations with the index finger. Sterile kaolin suspension (0.02ml using 0.25g/ml in sterile water) was slowly injected into the cisterna magna using a 27-gauge needle. Post-injection, the rats were monitored for about 1hr after which they were returned to their cages. The control rats had a sham saline injection into the cisterna magna. This technique has previously been established in the literature as a viable way of inducing hydrocephalus in experimental animals (Olopade *et al.*, 2021; Suryaningtyas *et al.*, 2019). Hydrocephalus was confirmed by the presence of a dome-shaped head (Chen *et al.*, 2017; Suryaningtyas *et al.*, 2019; Olopade *et al.*, 2021). Body weights of rats were measured twice a week. The general behaviour of the rats and their head circumference were observed daily. Rats with marked rapid weight loss, unsteady gait, or fatigue were isolated from the litter to prevent attack from their mates.

Post-Induction Protocol: The rats were euthanized at one-week post induction; two weeks post induction and four weeks post induction respectively and grouped as such. Euthanasia commenced with ketamine/xylazine (90/10mg/kg) anaesthesia followed by trans cardiac perfusion with normal saline to flush off the blood and then with 10% neutral buffered formalin. The brains were quickly collected and fixed in 10% neutral buffered formalin

for seven days. Coronal sections of the brains were made at the level of the optic chiasma and the diameter of the lateral ventricle at that level was measured in all the groups. Mild ventriculomegaly was defined as a ventricular diameter of less than 1.5mm, moderate ventriculomegaly as a ventricular diameter of more than 1.5mm with the cortex still opposed to the caudate/putamen and severe ventriculomegaly was defined as a visible separation of the cerebral cortex from the caudate/putamen with ventriculomegaly according to Olopade *et al.* (2021).

Histological Studies: A subset of the brains in each group underwent the Golgi staining technique of Smith and Roopar 2012 (as described by Sarkala *et al.*, 2023). The chromatin solution was prepared with 60 ml of 3% potassium dichromate solution and 20 ml of 10% formalin. The rat brains were transferred from the fixative into the chromatin solution and left in a dark place for two days. A second chromatin solution containing 60ml of 3g of potassium dichromate, 2 grams of chloral hydrate, and 5–7 drops of Dimethyl sulphoxide mixed in 100ml of distilled water and 20ml of 10% formalin. The tissues were transferred into the second chromatin solution and left for another 2 days. After the second day, this second chromatin solution was replaced with a fresh one. The tissues were left in the fresh chromatin solution for another three days. Next, the tissues were placed in a 0.75% silver nitrate solution containing 0.75g of silver nitrate dissolved in 100 ml of distilled water and left for three days in a row; the silver nitrate solution was changed daily. Tissue pieces were placed in a petri dish and the silver deposits were gently brushed off after the tissue pieces had been impregnated for 72 hours in silver nitrate. At this point, the tissue blocks were handled extremely delicately and cautiously. The tissues underwent a 10-minute dehydration process in absolute alcohol, followed by a 30-minute paraffin infiltration and subsequent embedding in paraffin wax. A rotary microtome then sectioned the tissue blocks at 60µm. Following a 10-minute soak in 70% alcohol, the sliced pieces were placed in xylene to become translucent, mounted on gelatin-coated glass slides, coverslipped with DPX, and left to dry for four days at room temperature. The brain samples of the experimental animals were bisected at the level of the optic chiasma. They were fixed, dehydrated, cleared, infiltrated, embedded and sectioned at 5µm. These sections were mounted on gelatin-coated slides and routinely stained with Haematoxylin and Eosin and Cresyl violet stains. The corpus callosum was measured in the midline at the level of the optic chiasma, midline. The sensorimotor cortex was quantitatively assessed by taking measures in the dorsolateral region (Femi-Akinlosotu *et al.*, 2019).

Histomorphometry: Using an Olympus CH (Japan) microscope connected to TS view analysis software at x40 magnification, the dendritic arbors of the pyramidal cells were visualised for the golgi stained sections. Only the layer V pyramidal neurons were subjected to light microscopic examination. Cortical and corpus callosum thicknesses were measured in the H&E stained slides of the control and experiment groups using the measurement module of the TS view image analysis software that came with the microscope.

Cell Count Analyses: Cell count analyses were performed on the Cresyl-stained slides using an Olympus CH image microscope connected to TS view analysis software and an X40 objective lens. Only visible nuclei were used to count cells. The following common standards were used to differentiate between pyramidal and non-pyramidal neurons: (1) Pyramidal neurons displayed a distinctive triangular shape with a single large apical dendrite. (2) Non-pyramidal neurons were distinguished by their smaller size and lack of the preceding criteria. An impartial counting frame measuring 1088 square meters was employed. The sampling field was methodically moved through the cortical layer of interest until the number of different types of pyramidal cells was determined.

Statistical analysis: Data collected were expressed as means $\pm$ SD and analyzed by one-way ANOVA test using GraphPad Prism Version 6.0 (GraphPad software, San Diego, California USA). The confidence interval was computed at the 95% level, and statistical significance was set at $p < 0.05$ for the null hypothesis being true by chance.

RESULTS

The study involved 197 rats, aged three weeks, in total. A kaolin suspension was injected intracisternal into 155 subjects, while 42 individuals served as controls. Twenty-seven rats died during the induction process, leaving 128 rats that made it through. A puncture to the brainstem, most

likely the respiratory centre, may have killed most of the rats; some may not have recovered consciousness following anaesthesia. Only 102 experimental rats—35 for the one-week study, 36 for the two-week study, and 31 for the four-week study—were utilized in the investigation after an additional 26 died two to three days after induction. About 70.5% of the induced 3-week-old rats in this study developed hydrocephalus.

Physical observation: The hydrocephalic rats exhibited clear hydrocephalic symptoms, such as an enlarged, dome-shaped head and an unsteady gait. After a week of kaolin injection, these characteristics were visible. They often experienced varied degrees of a hunched back, bloody discharge from the eye, and an unbalanced gait (Plate 1). When being handled, a few of them displayed aggressive behaviour.

Body weight: At one, two, and four weeks after induction, the hydrocephalic rats' weights were considerably lower than those of the age-matched controls (Fig. 1).

Gross Examination of the Brain: Upon close examination, the control brains' lateral ventricles were found to have slit-like spaces that are difficult for the unaided eye to see. The three categories of hydrocephalus—mild, moderate, and severe—were caused by different degrees of ventricular dilatation in the brains of the experimental rats.

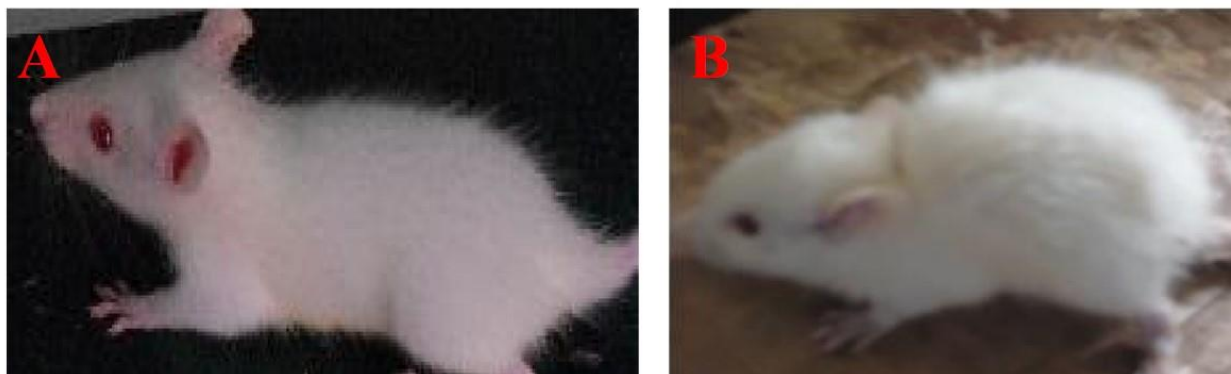


Plate 1

(A) Control rat; (B) Hydrocephalic rat. Note the hunched back and ruffled fur in the hydrocephalic rat.

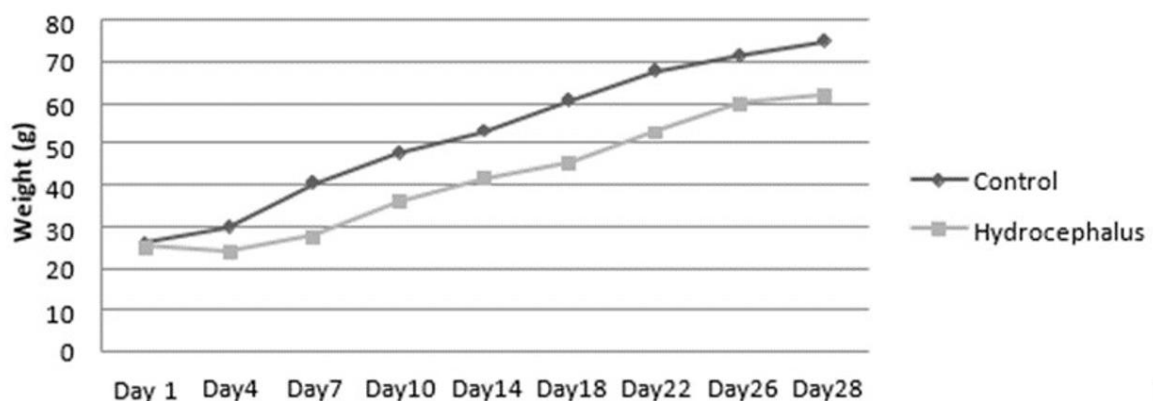


Figure 1:

Body weights of the hydrocephalic and control rats from induction until 4 weeks post-induction.

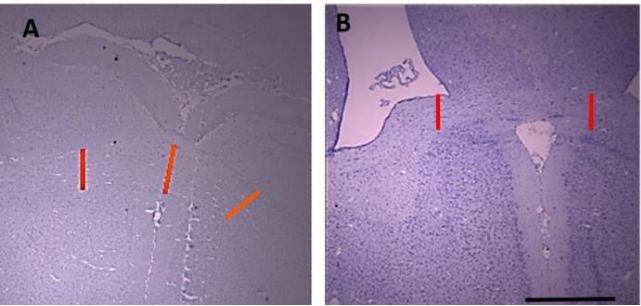
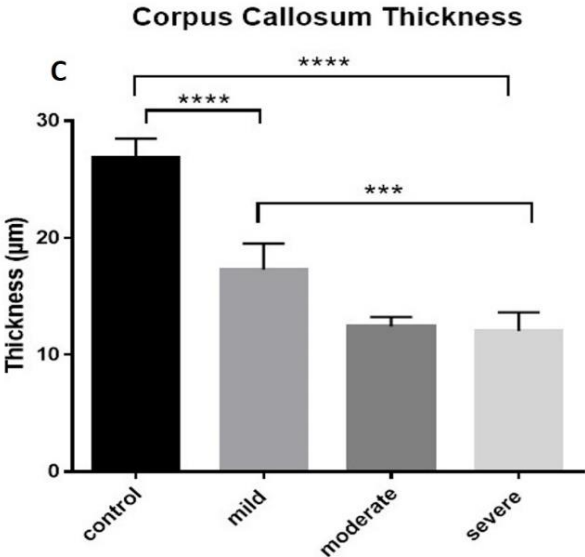


Figure 2: (A&B) Representative cresyl violet stained brain sections of control (A) and hydrocephalic (B) rats. Note the dimensions of the corpus callosum (red line) in both control and hydrocephalic sections. Scale bar: 50µm; (C) Thickness of the corpus callosum in the various category of hydrocephalus. The corpus callosum of the control rats was significantly thicker than the hydrocephalic groups ($p<0.0001^*$). The corpus callosum in mild hydrocephalic rats was significantly thicker when compared to moderate and severe hydrocephalic rats ($p=0.0064^{**}$).



Corpus callosum thickness: Hydrocephalic rats and control groups differed significantly ($p<0.0001$) in the thickness of their corpus callosum. Compared to moderate and severe hydrocephalic rats (control: $27\pm0.86\mu\text{m}$; mild: $17\pm1.10\mu\text{m}$; moderate: $12\pm0.40\mu\text{m}$; severe: $12\pm0.60\mu\text{m}$; $p=0.0064$ respectively), the corpus callosum of mild hydrocephalic rats was significantly thicker. The thickness of the corpus callosum did not, however, differ significantly between the moderate and severe hydrocephalic rats ($p=0.2384$) (Fig. 2C).

Cortical thickness of the sensorimotor cortex: Measuring the sensorimotor cortical thickness of both control ($109\pm3.30\mu\text{m}$) and hydrocephalic rats (mild: $65\pm1.30\mu\text{m}$; moderate: $61\pm0.73\mu\text{m}$; severe: $47\pm1.90\mu\text{m}$) showed a statistically significant difference ($p<0.0001$) between the control group and the various hydrocephalus categories, while there was also a significant difference ($p<0.0001$) between the various hydrocephalus categories. Rats with mild hydrocephalus had a significantly thicker cortical dimension than rats with moderate and severe hydrocephalus ($p=0.0302$ and $p<0.0001$, respectively). Rats with moderate hydrocephalus had a significantly thicker cortical dimension than those with severe hydrocephalus ($p<0.001$). (Figure 3).

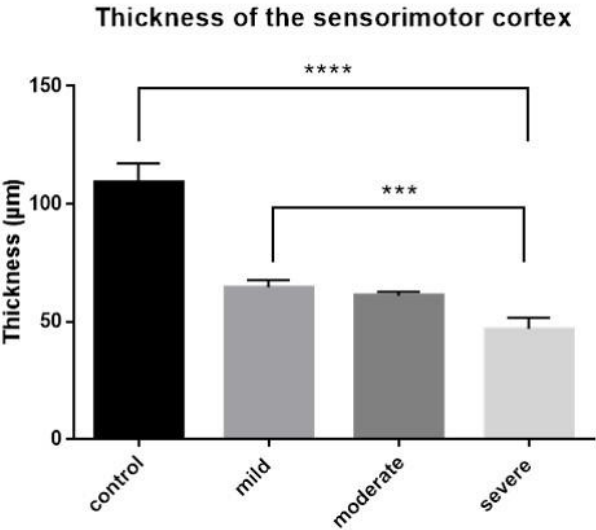


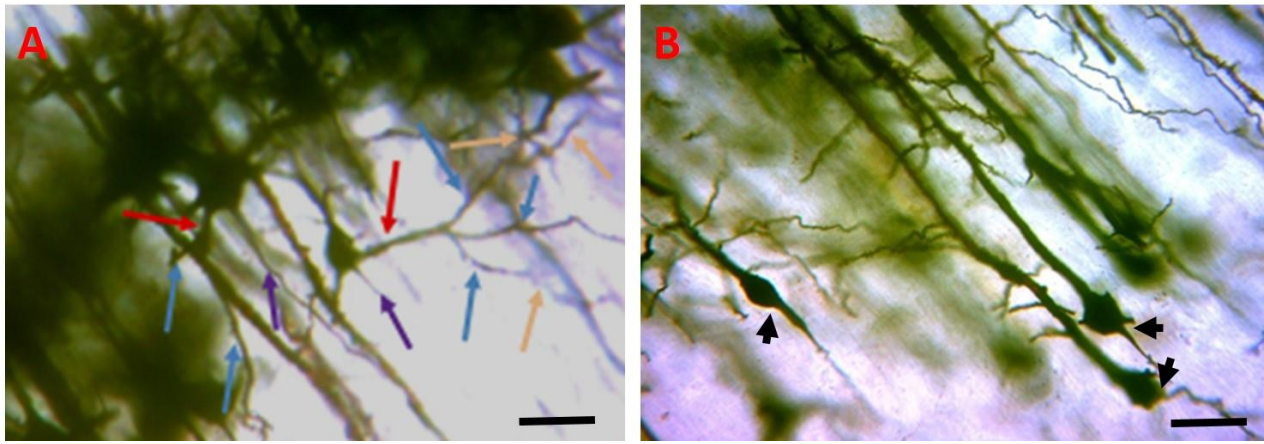
Figure 3: Thickness of the sensorimotor cortex in the various categories of hydrocephalus. The cortex in the control rats was significantly thicker than the hydrocephalic animals ($p<0.0001^*$). The cerebral cortex in mild hydrocephalic rats was significantly thicker when compared to moderate hydrocephalic rats ($p<0.05^{**}$). The cerebral cortex in moderate hydrocephalic rats was significantly thicker when compared to severe hydrocephalic rats ($p<0.001^{***}$).

Pyramidal neuron count: The pyramidal neuronal count in the hydrocephalic groups and that of their age-matched controls did not differ statistically significantly. ($p=0.419$; 0.162 ; 0.791) (Table 1).

Table 1: Pyramidal cell count in layer V of the control and hydrocephalic groups.

Group	n	Control	Hydrocephalic	p-value
1week	6	85.5±2.12	83.5±0.71	0.419
2 weeks	6	71.6± 2.88	61.7 ±6.8	0.075
4 weeks	6	86± 6.25	76 ±6.67	0.791

Golgi stain: Pyramidal cells were defined as those cells with pear-shaped cell bodies. Each soma gives rise to a single apical dendrite and a series of basal dendrites. The apical dendrite generally coursed to the cortical surface. The apical dendrites of the superficial neurons are shorter than the deeper neurons. The branching patterns of the basal dendrites in the experimental group were simple/ less complex when compared to the control groups (Plate 2).

**Plate 2:**

Photomicrographs of Golgi-impregnated layer V pyramidal neurons of (A) control rats (2weeks post-induction) having complex branching order (basal dendrites exhibited primary, secondary and tertiary branches) compared to (B) hydrocephalic rats (2weeks post-induction) showing denudation of the basal dendrite (black arrow-heads) (red arrow: primary basal dendrite; blue arrow: secondary basal dendrite, orange arrow: tertiary dendrite; purple arrow: axon; Scale bar: 10µm).

DISCUSSION

The injection of kaolin into the cisterna magna is a well-established induction model for experimental hydrocephalus leading to inflammation and fibrosis of the CSF pathways close to the fourth ventricle apertures and in the basal subarachnoid compartment (Femi-Akinlosotu *et al.*, 2019; Olopade *et al.*, 2021; Chen *et al.*, 2022; Parenrengi *et al.*, 2024). Some rats did not develop hydrocephalus at all, while others showed varying degrees of ventriculomegaly in response to an identical volume of kaolin injected into their brains for experimentation. Extracranial lymphatic vessels have been identified as a primary pathway for the absorption of CSF. It is believed that CSF travels from the subarachnoid space via the cribriform plate, the lymphatic vessels in the olfactory submucosa, and venous system at the base of the neck (Chae *et al.*, 2024; Spera *et al.*, 2023). Several investigators have reported variability in ventricular distension in different animal models with internal hydrocephalus (Catalão *et al.*, 2019; Czerwik *et al.*, 2023) and a relationship between CSF outflow resistance (through the extra-cranial lymphatic vessels) and ventricle size in kaolin-induced hydrocephalus models (Kaolin *et al.*, 2023; Nagra *et al.*, 2010; Spera *et al.*, 2023). Consequently, the varying degrees of ventriculomegaly might be due to the lowest lymphatic CSF absorption levels in relation to the highest degree of ventriculomegaly.

The hydrocephalic rats exhibited a hunched back, enlarged doom-shaped head, and an unsteady gait, all of which have been documented in other animal models of hydrocephalus caused by kaolin (Catalão *et al.*, 2019; Femi-Akinlosotu *et al.*, 2019; Chen *et al.*, 2022; Kaolin *et al.*, 2023; de Souza *et al.*, 2024; Parenrengi *et al.*, 2024). Gross examination of the induced rats' brains after sacrifice revealed varying degrees of ventriculomegaly even though some of them did not exhibit any overt symptoms of hydrocephalus.

The hydrocephalic rats showed initial weight loss and a gradual weight gain which have been reported as the earliest signs of hydrocephalus (Femi-Akinlosotu *et al.*, 2019; Olopade *et al.*, 2021). The hydrocephalic rats' weights were, nevertheless, consistently lower than the control groups

throughout the investigation. It was also noted that the hydrocephalic rats showed little to no appetite in the first 24 to 36 hours following induction; after that, their eating patterns returned to normal. Del Bigio *et al.* proposed two possible explanations: either hypothalamic regulation of pituitary function might be changed, or ventricular enlargement affected the hypothalamus's centres responsible for appetite (Del Bigio *et al.*, 2003). Anthropometric data from previous investigators revealed that children with hydrocephalus frequently have stunted growth, low weight, poor language, motor and social skills (Grace *et al.*, 2024; Perenc *et al.*, 2022; Rush *et al.*, 2022) and pituitary dysfunction has been suggested as a possible cause. Fetal-onset hydrocephalus had a substantial impact on the hypothalamic gonadotrophin-releasing hormone (Catalão *et al.*, 2019; McAllister *et al.*, 2007). Axons in the cerebral cortex or striatum may have stretched or neurons may have disconnected, resulting in a decline in motor skills and the unsteady gait seen in hydrocephalic rats (Olopade *et al.*, 2012; Chen *et al.*, 2017; de Oliveira & Fabris Vidal, 2020).

The hydrocephalic rats had thinner corpus callosum, which is consistent with previous studies (Olopade *et al.*, 2012; Jugé *et al.*, 2016; Pozzi *et al.*, 2021; Campos-Ordoñez *et al.*, 2023; Rodríguez-Pérez *et al.*, 2024). Stretching of the corpus callosum around the expanding ventricle results in disconnection of neurons, and atrophy of the corpus callosum is allegedly caused by a combination of mechanical injury, impaired blood flow, and accumulation of waste products in the CSF. In a hydrocephalic state, the cerebral cortex thickness indicates a state of compression (Olopade *et al.*, 2012; Jugé *et al.*, 2016; Campos-Ordoñez *et al.*, 2023). The hydrocephalic rats and controls' pyramidal neuron counts did not vary substantially across the age ranges. This suggests that the cortex was compressed using different techniques, excluding the loss of pyramidal neurons, particularly in layer V. A decrease in the extracellular water content and venous blood flow in the impacted cortex has been suggested as the cause of the compression (Olopade *et al.*, 2012; Jugé *et al.*, 2016; Chen *et al.*, 2017; Garcia-Bonilla *et al.*, 2022; Campos-Ordoñez *et al.*, 2023).

Layer V pyramidal cells, also called Betz cells, are large extra-telencephalic projection neurons that innervate α -motoneurons in the brainstem and spinal cord of primates and other mammalian species (Benavides-Piccione *et al.*, 2021; Nolan *et al.*, 2024). Although the hydrocephalic rats' basal dendrites primarily consist of primary basal dendrites with some denudation, the pyramidal neurons' basal dendrites in the control rats showed complex branching from primary to tertiary level. Femi-Akinlosotu *et al.* noted a similar effect in day-old hydrocephalic mice (Femi-Akinlosotu *et al.*, 2019). As the majority of dendritic branches form upon the arrival of thalamic fibers and other afferent fibers, the decrease in dendritic branching (basal dendrites) of pyramidal neurons may be the result of cortical de-afferentation. The basal dendrites that subcortical afferent fibers synapse on may have withdrawn or disconnected as a result of deafferentation. Alternatively, it may have been caused by the pressure of enlarged ventricles, which is first exerted on periventricular regions, affecting the basal dendrites of pyramidal neurons located deep in the cerebral cortex. The impact of hydrocephalus on the efferent axons could also be a possibility, as the trauma may impair axonal transmission by causing damage to the subcortical white matter. Because efferent connectivity to subcortical structures and the contralateral cortex is established by postnatal day 1 and predates the arrival of afferent connections, it seems unlikely that any dendritic abnormality was caused by the deafferentation of efferent axons. The direct result of the hydrocephalic process may then be the effect of hydrocephalus on the basal dendrite of pyramidal neurons, the primary efferent neurons of the cortex.

The postnatal stage of brain development is essential for the proper expression of brain-related functions (Soch *et al.*, 2020; Kourosh-Arabi *et al.*, 2021). In terms of brain development, the 3-week-old rats used in this study are about the same age as a 6-month-old human infant (Sengupta, 2013; Zeiss, 2021). Age-related increases in brain plasticity occur as development proceeds, and developmental assaults on brain tissue can result in functional deficits. According to our research, the degree of ventriculomegaly associated with hydrocephalus altered the pyramidal neurons' basal dendrites and reduced the thickness of the cerebral cortical and corpus callosum. Although we could only qualitatively evaluate the dendritic arborization of the pyramidal neurons and there was no imaging method available to evaluate the rats' hydrocephalic status before sacrifice, we think the data from this study will serve as a foundation for future investigations into experimental hydrocephalus studies. Since neural networks are critical for survival, this research could lead to a better understanding of the circuitry configurations in the cerebral cortex and, consequently, a better understanding of the pathophysiology of juvenile hydrocephalus in experimental rats. The ultrastructure and mode of action of these synaptic connections in experimental juvenile hydrocephalic states, however, require more investigation.

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REFERENCES

- Benavides-Piccione, R., Blazquez-Llorca, Kastanauskaite, A., Fernaud, I., Gonzalez-Tapia, S., DeFelipe, J., 2024. Key morphological features of human pyramidal neurons. *bioRxiv* 2023.11.10.566540. <https://doi.org/10.1101/2023.11.10.566540>
- Benavides-Piccione, R., Rojo, C., Kastanauskaite, A., Defelipe, J., 2021. Variation in Pyramidal Cell Morphology Across the Human Anterior Temporal Lobe. *Cerebral Cortex* 31, 3592–3609. <https://doi.org/10.1093/CERCOR/BHAB034>
- Campos-Ordoñez, T., González-Granero, S., Eudave-Patiño, M., Buriticá, J., Herranz-Pérez, V., García-Verdugo, J.M., Gonzalez-Perez, O., 2023. Normal pressure hydrocephalus decreases the proliferation of oligodendrocyte progenitor cells and the expression of CNPase and MOG proteins in the corpus callosum before behavioral deficits occur. *Exp Neurol* 365, 114412. <https://doi.org/10.1016/J.EXPNEUROL.2023.114412>
- Catalão, C.H.R., Souza, A.O., Santos-Júnior, N.N., da Silva, S.C., da Costa, L.H.A., Alberici, L.C., Rocha, M.J.A., da Silva Lopes, L., 2019. Kaolin-induced hydrocephalus causes acetylcholinesterase activity dysfunction following hypothalamic damage in infant rats. *Brain Res* 1724, 146408. <https://doi.org/10.1016/J.BRAINRES.2019.146408>
- Chae, J., Choi, M., Choi, J., Yoo, S.J., 2024. The nasal lymphatic route of CSF outflow: implications for neurodegenerative disease diagnosis and monitoring. *Anim Cells Syst* (Seoul) 28, 45–54. <https://doi.org/10.1080/19768354.2024.2307559>
- Chen, L.J., Chen, J.R., Tseng, G.F., 2022. Modulation of striatal glutamatergic, dopaminergic and cholinergic neurotransmission pathways concomitant with motor disturbance in rats with kaolin-induced hydrocephalus. *Fluids Barriers CNS* 19, 1–14. <https://doi.org/10.1186/S12987-022-00393-1/FIGURES/8>
- Chen, L.J., Wang, Y.J., Chen, J.R., Tseng, G.F., 2017. Hydrocephalus compacted cortex and hippocampus and altered their output neurons in association with spatial learning and memory deficits in rats. *Brain Pathology* 27, 419. <https://doi.org/10.1111/BPA.12414>
- Curzio, D.L. Di, Curzio, D.L. Di, 2017. Neuropathological Changes in Hydrocephalus—A Comprehensive Review. *Open Journal of Modern Neurosurgery* 8, 1–29. <https://doi.org/10.4236/OJMN.2018.81001>
- Czerwik, A., Schmidt, M.J., Olszewska, A., Hinz, S., Büttner, K., Farke, D., 2023. Reliability and interobserver variability of a grading system of ventricular distension in dogs. *Front Vet Sci* 10, 1271545. <https://doi.org/10.3389/FVETS.2023.1271545/BIBTEX>
- de Oliveira, G.R., Fabris Vidal, M., 2020. A normal motor development in congenital hydrocephalus after Cuevas Medek Exercises as early intervention: A case report. *Clin Case Rep* 8, 1226–1229. <https://doi.org/10.1002/CCR3.2860>
- de Souza, S.N.F., Machado, H.R., da Silva Lopes, L., da Silva Beggiora Marques, P., da Silva, S.C., Dutra, M., Aragon, D.C., Santos, M.V., 2024. Evaluation of the behavioral, histopathological, and immunohistochemical effects resulting from ventriculosubcutaneous shunt obstruction in kaolin-induced hydrocephalus in rats. *Child's Nervous System* 40, 1533–1539. <https://doi.org/10.1007/S00381-023-06260-0/METRICS>
- Del Bigio, M.R., 2010. Neuropathology and structural changes in hydrocephalus. *Dev Disabil Res Rev* 16, 16–22. <https://doi.org/10.1002/DDRR.94>

- Del Bigio, M.R., Wilson, M.J., Enno, T., 2003. Chronic hydrocephalus in rats and humans: White matter loss and behavior changes. *Ann Neurol* 53, 337–346. <https://doi.org/10.1002/ANA.10453>
- Femi-Akinlosotu, O.M., Shokunbi, M.T., Naicker, T., 2019. Dendritic and synaptic degeneration in pyramidal neurons of the sensorimotor cortex in neonatal mice with kaolin-induced hydrocephalus. *Front Neuroanat* 13, 426791. <https://doi.org/10.3389/FNANA.2019.00038/BIBTEX>
- Galakhova, A.A., Hunt, S., Wilbers, R., Heyer, D.B., de Kock, C.P.J., Mansvelder, H.D., Goriounova, N.A., 2022. Evolution of cortical neurons supporting human cognition. *Trends Cogn Sci* 26, 909–922. <https://doi.org/10.1016/J.TICS.2022.08.012>
- Garcia-Bonilla, M., Castaneyra-Ruiz, L., Zwick, S., Talcott, M., Otun, A., Isaacs, A.M., Morales, D.M., Limbrick, D.D., McAllister, J.P., 2022. Acquired hydrocephalus is associated with neuroinflammation, progenitor loss, and cellular changes in the subventricular zone and periventricular white matter. *Fluids Barriers CNS* 19, 1–18. <https://doi.org/10.1186/S12987-022-00313-3/FIGURES/7>
- Grace, N., Mbabazi, E., Mukunya, D., Tumuhameye, J., Okechi, H., Wegoye, E., Olupot-Olupot, P., Matovu, J.K., Hopp, L., Napyo, A., 2024. High burden of wasting among children under-five with hydrocephalus receiving care at CURE children's hospital in Uganda: a cross-sectional study. *BMC Nutr* 10, 1–10. <https://doi.org/10.1186/S40795-024-00819-Z/TABLES/2>
- Hunt, S., Leibner, Y., Mertens, E.J., Barros-Zulaica, N., Kanari, L., Heistek, T.S., Karnani, M.M., Aardse, R., Wilbers, R., Heyer, D.B., Goriounova, N.A., Verhoog, M.B., Testa-Silva, G., Obermayer, J., Versluis, T., Benavides-Piccione, R., De Witt-Hamer, P., Idema, S., Noske, D.P., Baayen, J.C., Lein, E.S., Defelipe, J., Markram, H., Mansvelder, H.D., Schürmann, F., Segev, I., De Kock, C.P.J., 2023. Strong and reliable synaptic communication between pyramidal neurons in adult human cerebral cortex. *Cerebral Cortex* 33, 2857–2878. <https://doi.org/10.1093/CERCOR/BHAC246>
- Jugé, L., Pong, A.C., Bongers, A., Sinkus, R., Bilston, L.E., Cheng, S., 2016. Changes in Rat Brain Tissue Microstructure and Stiffness during the Development of Experimental Obstructive Hydrocephalus. *PLoS One* 11, e0148652. <https://doi.org/10.1371/JOURNAL.PONE.0148652>
- Kahle, K.T., Klinge, P.M., Koschnitzky, J.E., Kulkarni, A. V., MacAulay, N., Robinson, S., Schiff, S.J., Strahle, J.M., 2024. Paediatric hydrocephalus. *Nature Reviews Disease Primers* 2024 10:1 10, 1–16. <https://doi.org/10.1038/s41572-024-00519-9>
- Kahle, K.T., Kulkarni, A. V., Limbrick, D.D., Warf, B.C., 2016. Hydrocephalus in children. *The Lancet* 387, 788–799. [https://doi.org/10.1016/S0140-6736\(15\)60694-8](https://doi.org/10.1016/S0140-6736(15)60694-8)
- Kaolin, D., Kan, O., Ile, E., Hidrosefali, İ., Aquaporin, Ü., İntrakraniyal, E., Ölçümü, B., Hayvan, A., Elif, D., Gündoğdu, B., Aydın, H.E., Avci Küpeli, Z., 2023. Intracranial Pressure Measurement of and Investigation of the Effect of Aquaporin on Hydrocephalus Induced by Experimental Kaolin and Autologous Blood Injection: An Animal Study. *Türkiye Klinikleri J Med Sci* 43, 149–59. <https://doi.org/10.5336/medsci.2022-93849>
- Kourosh-Arami, M., Hosseini, N., Komaki, A., 2021. Brain is modulated by neuronal plasticity during postnatal development. *Journal of Physiological Sciences* 71, 1–16. <https://doi.org/10.1186/S12576-021-00819-9/TABLES/1>
- Maller, V. V., Gray, R.I., 2016. Noncommunicating Hydrocephalus. *Seminars in Ultrasound, CT and MRI* 37, 109–119. <https://doi.org/10.1053/J.SULT.2015.12.004>
- McAllister, J.P., Abdolvahabi, R.M., Walker, M.L., Mitchell, J.A., Jones, H.C., 2007. Effects of congenital hydrocephalus on the hypothalamic gonadotrophin-releasing hormone system. *Neurosurg Focus* 22, 1–10. <https://doi.org/10.3171/FOC.2007.22.4.5>
- Nagra, G., Wagshul, M.E., Rashid, S., Li, J., McAllister, J.P., Johnston, M., 2010. Elevated CSF outflow resistance associated with impaired lymphatic CSF absorption in a rat model of kaolin-induced communicating hydrocephalus. *Cerebrospinal Fluid Res* 7, 4. <https://doi.org/10.1186/1743-8454-7-4>
- Nolan, M., Scott, C., Hof, P.R., Ansorge, O., 2024a. Betz cells of the primary motor cortex. *J Comp Neurol* 532, 1–29. <https://doi.org/10.1002/CNE.25567>
- Nolan, M., Scott, C., Hof, P.R., Ansorge, O., 2024b. Betz cells of the primary motor cortex. *J Comp Neurol* 532, 1–29. <https://doi.org/10.1002/CNE.25567>
- Olopade, F.E., Femi-Akinlosotu, O., Adekanmbi, A.J., Ajani, S., Shokunbi, M.T., 2021. Neurobehavioural changes and morphological study of cerebellar purkinje cells in kaolin induced hydrocephalus. *Anat Sci Int* 96, 87–96. <https://doi.org/10.1007/S12565-020-00561-Z/METRCS>
- Olopade, F.E., Shokunbi, M.T., Sirén, A.L., 2012. The relationship between ventricular dilatation, neuropathological and neurobehavioural changes in hydrocephalic rats. *Fluids Barriers CNS* 9, 19. <https://doi.org/10.1186/2045-8118-9-19>
- Parentrengi, M.A., Dariansyah, A.D., Suryaningtyas, W., Fauziah, D., Sudiana, I.K., Utomo, B., Gunawan, P.I., 2024. Effects of Cerebrospinal Fluid Drainage on Pro-Inflammatory and Anti-Inflammatory Cytokines Expression in the Subventricular Zone of Kaolin-Induced Hydrocephalic Rats. *Pharmacognosy Journal* 16, 20–27. <https://doi.org/10.5530/pj.2024.16.3>
- Perenc, L., Guzik, A., Podgórska-Bednars, J., Drużbicki, M., 2022. Somatic Development Disorders in Children and Adolescents Affected by Syndromes and Diseases Associated with Neurodysfunction and Hydrocephalus Treated/Untreated Surgically. *International Journal of Environmental Research and Public Health* 2022, Vol. 19, Page 5712 19, 5712. <https://doi.org/10.3390/IJERPH19095712>
- Pozzi, N.G., Brumberg, J., Todisco, M., Minafra, B., Zangaglia, R., Bossert, I., Trifirò, G., Ceravolo, R., Vitali, P., Isaías, I.U., Fasano, A., Pacchetti, C., 2021. Striatal Dopamine Deficit and Motor Impairment in Idiopathic Normal Pressure Hydrocephalus. *Movement Disorders* 36, 124–132. <https://doi.org/10.1002/MDS.28366>
- Rekate, H.L., Blitz, A.M., 2016. Hydrocephalus in children. *Handb Clin Neurol* 136, 1261–1273. <https://doi.org/10.1016/B978-0-444-53486-6.00064-8>
- Rodríguez-Pérez, L.M., López-de-San-Sebastián, J., de Diego, I., Smith, A., Roales-Buján, R., Jiménez, A.J., Paez-Gonzalez, P., 2024. A selective defect in the glial wedge as part of the neuroepithelium disruption in hydrocephalus development in the mouse hyh model is associated with complete corpus callosum dysgenesis. *Front Cell Neurosci* 18, 1330412. <https://doi.org/10.3389/FNCEL.2024.1330412/BIBTEX>
- Rush, J., Pa'la, A., Kapapa, T., Wirtz, C.R., Mayer, B., Micah-Bonongwe, A., Gladstone, M., Kamalo, P., 2022. Assessing neurodevelopmental outcome in children with hydrocephalus in Malawi. A pilot study. *Clin Neurol Neurosurg* 212, 107091. <https://doi.org/10.1016/J.CLINEURO.2021.107091>
- Sarkala, H.B., Jahanshahi, M., Dolatabadi, L.K., Namavar, M.R., 2023. G-CSF improved the memory and dendritic

- morphology impairments in the hippocampal CA1 pyramidal neurons after brain ischemia in the male rats. *Metab Brain Dis* 38, 2573–2581. <https://doi.org/10.1007/S11011-023-01286-4/METRICS>
- Sengupta, P., 2013. The Laboratory Rat: Relating Its Age with Human's. *Int J Prev Med* 4, 624.
- Soch, A., Sominsky, L., Younesi, S., De Luca, S.N., Gunasekara, M., Bozinovski, S., Spencer, S.J., 2020. The role of microglia in the second and third postnatal weeks of life in rat hippocampal development and memory. *Brain Behav Immun* 88, 675–687. <https://doi.org/10.1016/J.BBI.2020.04.082>
- Spera, I., Cousin, N., Ries, M., Kedracka, A., Castillo, A., Aleandri, S., Vladymyrov, M., Mapunda, J.A., Engelhardt, B., Luciani, P., Detmar, M., Proulx, S.T., 2023. Open pathways for cerebrospinal fluid outflow at the cribriform plate along the olfactory nerves. *EBioMedicine* 91. <https://doi.org/10.1016/J.EBIOM.2023.104558>
- Suryaningtyas, W., Arifin, M., Rantam, F.A., Bajamal, A.H., Dahlan, Y.P., Dewa Gede Ugrasena, I., Maliawan, S., 2019. Erythropoietin protects the subventricular zone and inhibits reactive astrogliosis in kaolin-induced hydrocephalic rats. *Child's Nervous System* 35, 469–476. <https://doi.org/10.1007/S00381-019-04063-W/METRICS>
- Zeiss, C.J., 2021. Comparative Milestones in Rodent and Human Postnatal Central Nervous System Development. *Toxicol Pathol* 49, 1368–1373. https://doi.org/10.1177/01926233211046933/ASSET/IMA-GES/LARGE/10.1177_01926233211046933-FIG1.JPEG

Full length Research Article

Anti-arthritis Activity of Methanol Extract of *Peperomia pellucida* in Complete Freund's Adjuvant Induced Arthritis in Wistar Rats

* Uwaya O.D., Ohonbamu A.K. and Obasuyi E.I.

Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City

Summary: Plants in the Piperaceae family include *Peperomia pellucida*. It has been eaten and used to treat illnesses. This study tested the anti-arthritis activity of a 70% methanol extract of *Peperomia pellucida*. A complete Freund's adjuvant-induced arthritis animal model was used. Oral distilled water (10 ml/kg) did not cause arthritis in Group 1. In groups 2-4, arthritis was induced and treated with 100, 200, and 400 mg/kg of a 70% methanol extract of *Peperomia pellucida*. Group 5 received oral methotrexate (0.7 mg/kg) for arthritis pain. Group 6 received distilled water (10 ml/kg) for arthritis and was untreated. All inflamed animals' paw diameters, organ-to-body weight ratio, percentage change in body weight, erythrocyte sedimentation rate, haematological indices, biochemical parameters, and superoxide dismutase enzyme activity were measured. Additionally, joint and organ histopathology were examined. The 70% methanol extract of *Peperomia pellucida* significantly reduced arthritis inflammation diameter ($***p < 0.001$) compared to arthritis control. The weight change in extract-treated animals was not significant ($p > 0.5$) compared to healthy controls. Compared to the healthy control group, animals treated with the extract had no significant change ($p > 0.5$) in hematological and biochemical parameters. The extract and standard drug significantly reduced the erythrocyte sedimentation rate ($**p < 0.01$) compared to the arthritis control group. The extract increased superoxide dismutase enzyme activity in arthritis animals ($**p < 0.01$) compared to controls. Bone damage was significantly reduced in extract-treated animals compared to arthritis controls. Methanol extract of *Peperomia pellucida* increased endogenous superoxide dismutase and possessed anti-arthritis activity.

Keywords: *Peperomia pellucida*, superoxide dismutase, arthritis, aspartate aminotransferase.

*Authors for correspondence: dickson.uwaya@uniben.edu, Tel: +234- 705-965-0758

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INTRODUCTION

Phytomedicine has been useful in the treatment and management of diseases in humans. The use of herbal medicine dates back to the origin of the human race (Petrovska, 2012). Widely used herbal medicines have been synthesized from the therapeutic experience of generations of practicing physicians of ancient systems of medicine (Khopade and Vispute, 2011). Plant parts, as well as root, stem, and leaf extracts and polyherbal preparations, are administered to treat and manage numerous disorders (Choudhary *et al.*, 2015). Research into herbal drugs is of growing interest in order to develop alternatives to orthodox medicines that may produce fewer adverse effects and be more cost effective (Badami *et al.*, 2004). *Peperomia pellucida* is a common annual weed native to tropical North, Central, and South America, where, till date, the largest diversity has been encountered. Many species are endemic to the Andes and Amazon regions. It is now pantropical in distribution and is abundantly available in tropical and subtropical regions worldwide, including Angola, Zambia,

and Nigeria. The plant flourishes in loose and humid soil in moist and shady areas. *P. pellucida* is characterized by its smooth, fleshy, and heart-shaped leaves, succulent stems, shallow roots, and minute flowers, which eventually grow into numerous tiny seeds attached to the cord-like spikes (Frenzke *et al.*, 2015; Mosango, 2008). The current study evaluates the anti-arthritis activity of *Peperomia pellucida* using complete Freund's adjuvant induced arthritis in an animal model.

MATERIALS AND METHODS

Collection of Plant Material: The plant, *Peperomia pellucida*, was collected from humid farmland in Ogbeson, Ikpoba Hill, Benin City, from November to December, 2020. It was identified by Dr. H. A. Akinnibosun, a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

Extraction of Plant: The root of the plant was isolated from the whole plant and discarded. 95 g of plant was weighed and thoroughly washed using distilled water. The plant was then blended with 300 ml of distilled water using a blender. Afterwards, the mixture was dissolved in 700 ml of methanol and left to stand for three days. At the completion of the duration, the plant was sieved using cheesecloth, and the filtrate was concentrated over a water bath. The extract was stored in a refrigerator before use.

Animal Design: Thirty healthy Wistar rats of both sexes weighing between 205-300 g were purchased from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. The animals were housed within the animal house facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City. They were acclimatized for one week amid ideal environmental conditions. All animals were adequately fed with grower pellets and given potable water. The animals were handled according to standard protocol for laboratory animals (National Institute of Health, USA; Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002) and the ethical committee of the Department of Science and Laboratory Technology, Faculty of Life Sciences, University of Benin.

Anti-Arthritis Activity Of 70% Methanol Extract of *Peperomia pellucida* in Complete Freund Adjuvant (CFA) Induced Arthritis: Thirty (30) Wistar rats of both sexes weighing between 205-300 g were divided into six groups of four animals per group. In Group 1, the healthy control group, distilled water (10 ml/kg) was administered orally daily, and there was no induction of arthritis. Groups 2-4 received 100 mg/kg, 200 mg/kg, and 400 mg/kg of a 70% methanol extract of *Peperomia pellucida*, respectively, with arthritis induced. In Group 5, the standard group, methotrexate (0.7 mg/kg) was administered orally with arthritis-induced pain. In Group 6, the arthritis control group, arthritis was induced, and animals were left untreated. Before treatment, arthritis was induced in the animals in groups 2-6 by injecting a single dose of 0.1 ml of complete Freund's adjuvant (CFA) into the foot pad of the left hind paw of the animals and observed for 9 days. Complete Freund's adjuvant contains 1 mg/ml of dry, heat-killed *Mycobacterium tuberculosis* dissolved in sterile paraffin oil. Treatment was administered orally for 14 days after day 9 (Uwaya et al., 2022; Fatima and Fatima, 2016).

Arthritis Assessment: The paw size reading of left hind limb, 2cm below ankle, was taken on days 0, 2, 6, 10 and 14. This was done using a Digital Vernier Caliper (Mitutoyo, Japan) on all animals. Paw diameter was compared to baseline values obtained before induction of CFA.

Body Weight Assessment and Measurement of Organ Weights: The weights of the animals were taken on days 1 and 14 of the experiment before sacrifice. After sacrifice, the liver, kidney, lungs, heart, and spleen were discreetly removed and weighed using an analytical weighing balance. Percentage weight change and organ to body weight ratio were calculated using the formulas below:

Percentage weight change (%) = [(final weight - initial weight) / final weight] x 100
Organ-to-body weight ratio = weight of internal organ / final body weight

Determination of Erythrocyte Sedimentation Rate Using Wistar Green Method: On day 14, animals were sacrificed by anesthetizing with chloroform and dissected. Blood was collected from the abdominal aorta. 1 ml of blood was transferred into a Wistar green disposable ESR tube, 1 ml into an EDTA container, and the remaining volume was transferred into a plain container. The blood in the disposable ESR tube was allowed to stand for 1 hour, and the reading was taken.

Determination of Haematological Parameters: The blood in the EDTA bottle was used to analyze haematological parameters using an automated machine model (PCE-2100, Japan).

Determination of Biochemical Parameters: Blood collected in plain sample bottles without anticoagulant was centrifuged for 5 minutes (4900 rpm), and the serum was collected as a sample for assessment of biochemical parameters.

Determination of Alkaline Phosphatase (ALP): Alkaline phosphate substrate (0.5 ml) was pipetted into test tubes labeled blank, standard, and sample, respectively. 50 ul of distilled water, a standard, and a sample were added to the test tubes, respectively, and incubated for 10 minutes at 37 °C. Then, 2.5 ml of alkaline phosphatase colour developer was added to labeled test tubes. The spectrophotometer was zeroed with reagent blank, and the absorbencies of all tubes were measured at a wavelength of 590 nm. The concentration of alkaline phosphate was calculated using the following formula:

Alkaline Phosphatase Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

Determination of Alanine Aminotransferase (ALT): To 5 ml of each blood sample, 0.1 ml of solution RI (buffer) was added and mixed. It was incubated for 30 minutes at 37 °C. 0.5 ml of solution R2 (2,4-dinitrophenylhydrazine) was added, and the mixture was allowed to stand for 20 minutes at room temperature, after which 5.0 ml of sodium hydroxide was added. The process was repeated for all samples. The samples were read using a spectrophotometer at a wavelength of 546 nm. The samples were read against a blank.

Determination of Aspartate aminotransferase (AST): To 5 ml of each of the blood samples, 0.1 ml of solution RI (buffer) was added, mixed, and incubated for 30 minutes at 37 °C. Solution R2; 2,4-dinitrophenylhydrazine (0.5 ml) was added, and the mixture was allowed to stand for 20 minutes at room temperature, after which 5.0 ml of sodium hydroxide was added. The process was repeated for all samples. The samples were read using a spectrophotometer at a wavelength of 546 nm.

Determination of Total Protein: To 0.02 ml of the blood samples, distilled water and standard were pipetted into separate test tubes and labeled sample, blank, and standard,

respectively. To the test tube containing the sample and standard, 0.5 ml of solution R1 was added, and 0.5 ml of solution R2 was added to the test tube containing the blank. The solution in the test tube was then mixed and incubated for 30 minutes at 25 °C. The samples were read using a spectrophotometer at a wavelength of 546 nm. The samples were read against the blank, which contained both solutions and sodium hydroxide and water for absorbance. The concentration of total protein was calculated using the following formula:

Total Protein Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

Determination of Superoxide Dismutase (SOD): Carbonate buffer (2.5 ml) was measured into labelled test tubes. 0.2 ml of tissue homogenate was added to the test tubes. 0.2 ml of distilled water was also measured in the reference test tube. 0.3 ml of epinephrine solution was added to each of the test tubes and to the reference tube. They were properly mixed and read at an absorbance of 420 nm every 30 – 120 seconds with a UV spectrophotometer (Model T80+UV spectrophotometer, PG Instruments Ltd.). Distilled water was used to zero the machine (Misra and Fridouich, 1972).

Histopathological Assessment: After sacrifice, the left hind paws were severed discreetly. Tissue specimens were dehydrated in alcohol, processed in xylene, and then embedded in paraffin wax before sectioning was done using a microton. Sections of the tissues 5 µm thick were stained with hematoxylin and eosin (H & E) for viewing. All sections were viewed under a Leica R light microscope (Model DM500) at x100 and x400 magnification by a

histopathologist who was oblivious to the experimental grouping and protocol.

Statistical Analysis: Data are expressed as mean ± standard error of mean (SEM) in this study. Multiple comparisons of data were done using one-way ANOVA. $P > 0.5$ was consistent with non-significant values. The analysis of all the data was done using the software program GraphPad Prism.

RESULTS

Anti-arthritis effect of 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund adjuvant induced arthritis in Wistar rats

Table 1 shows the effect of 14-day daily oral administration of 70 % methanol extract of *Peperonia pellucidain* paw diameter in complete Freund's adjuvant induced arthritis in Wistar rats. Seventy percent methanol extract of *Peperonia pellucidain* significantly reduced ($***p < 0.001$) the diameter of inflammation of arthritis compared to arthritis control rats.

Organ-to-body weight ratio and percentage change in body weight following 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund's adjuvant induced arthritis in Wistar rats: Table 2 shows the organ to body weight ratio and percentage change in body weight following 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund's adjuvant induced arthritis in wistar rats. In the animals treated with the extract, change in weight was not significant ($p > 0.05$) compared to animals belonging to the healthy control group.

Table 1:

The anti-arthritis effect of 14-day daily oral administration of 70 % methanol extract of *Peperonia pellucidain* complete Freund adjuvant induced arthritis in Wistar rats

Dose (mg/kg)	Diameter (mm)				Inhibition (%)
	1 st	2 nd	3 rd	4 th	
Control	0.76 ± 0.15	0.66 ± 0.19***	0.91 ± 0.13***	0.72 ± 0.14****	73.43
100	2.20 ± 0.25*	2.00 ± 0.06**	1.51 ± 0.08*	1.32 ± 0.08**	51.29
200	2.51 ± 0.21**	1.89 ± 0.12**	1.48 ± 0.12**	1.22 ± 0.16***	54.98
400	2.82 ± 0.21**	1.77 ± 0.20***	1.45 ± 0.21**	1.11 ± 0.24***	59.04
STD	2.41 ± 0.51*	2.18 ± 0.14*	1.19 ± 0.21**	1.17 ± 0.38***	56.83
Arthritis alone	3.04 ± 0.34***	3.13 ± 0.27	2.78 ± 0.45	2.71 ± 0.07	-

The 70% methanol extract of *Peperonia pellucidain* significantly decreased the diameter of inflammation of arthritis at 100 mg/kg ($p > 0.01$) and all other dose levels ($p > 0.001$) as compared to the arthritis alone control group. Values are represented as mean ± SEM, $n = 4$ per group.

Table 2:

Organ-to-body weight ratio following 14-day daily oral administration of 70% methanol extract of *Peperonia pellucidain* complete Freund adjuvant induced arthritis in Wistar rats.

Dose (mg/kg)	H:BW (10^{-3})	Lu:BW (10^{-3})	S:BW (10^{-3})	Li:BW (10^{-3})	K:BW (10^{-3})	% Body Weight Change (%)
Control	3.2±0.48	6.5±0.96	3.3±0.48	3.3±0.46	4.3±0.48	0.69 ± 0.43
100	4.0±0.00	7.0±0.58	3.5±0.29	3.3±0.17	5.8±0.25	9.75 ± 4.96
200	3.3±0.25	6.3±0.25	3.3±0.25	3.2±0.70	5.0±0.00	3.75 ± 1.65
400	3.3±0.25	6.3±0.25	3.3±0.25	3.1±0.96	5.3±0.25	2.65 ± 2.27
Standard	3.5±0.50	8.5±0.25	2.8±0.48	2.4±0.65	6.5±0.87	6.35 ± 1.39
Arthritis alone	4.0±0.41	1.1±2.60	3.5±0.29	2.8±0.75	5.8±0.25	6.13 ± 2.20

Test groups show no significant difference ($p > 0.5$) in organ-to-body weight ratio and no significant difference ($p > 0.05$) in percentage weight change compared to the arthritis-only control group. Values are represented as mean ± SEM, $n = 4$ per group.

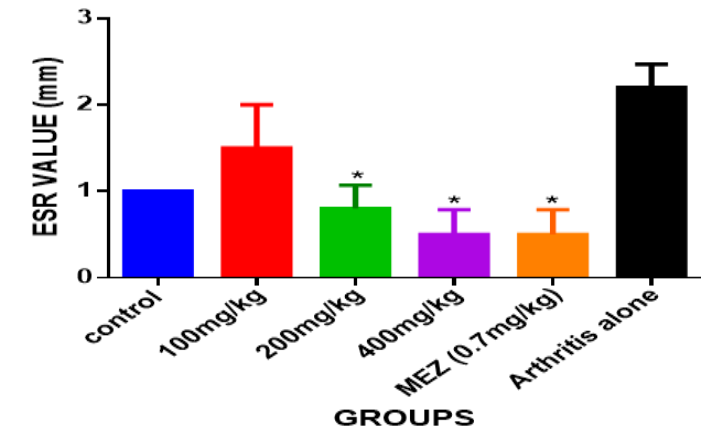


Figure 1
The effect of 14 days daily oral administration of a 70% methanol extract of *Peperomia pellucida* on erythrocyte sedimentation rate in complete Freund adjuvant induced arthritis in Wistar rats. There was a significant decrease (** $p > 0.01$) in erythrocyte sedimentation rate at 200 mg/kg and 400 mg/kg dose levels and in the standard group as compared to the arthritis-only control group. Values are represented as mean $\pm$ SEM, $n = 4$ per group

Table 3:
The effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on haematological parameters in complete Freund adjuvant induced arthritis in Wistar rats

DOSE(mg/kg)	WBC (10 ³ /uL)	LYM (10 ³ /uL)	MID (10 ³ /uL)	GRAN (10 ³ /uL)	RBC (10 ⁶ /uL)	HGB (g/dL)	HCT (%)	PLT (10 ³ /uL)	PCT (%)
Control	9.2±0.27	83.4±1.10	12.1±0.82	4.5±0.29	7.5±0.10	14.3±0.22	44.0±0.53	629.3±66.2	0.46± 0.06
100	12.6±0.94	77.6±8.95	16.4±6.14	6.0±2.82	6.7±0.73	12.9±1.47	40.6±3.47	839.0±202.10	0.6±0.17
200	13.4±0.90	82.9±3.32	11.3±2.17	5.8±1.17	7.7±0.10	15.0±0.26	45.0±0.68	650.0±12.33	0.5± 0.01
400	13.2±1.49	80.6±2.87	13.5±1.86	5.9±1.17	7.8±0.07	15.1±0.25	44.9±1.34	879.0± 160.30	0.8± 0.18
Standard	12.9±0.51	72.9±6.93	17.4±3.52	9.7± 3.45	7.0±0.10	14.0±0.28	41.3±1.05	759.80±55.47	0.62±0.08
Arthritis alone	10.63±1.37	83.6±3.97	10.2±2.72	6.23±1.73	7.3±0.12	13.6±0.31	43.7±1.58	642.5±59.20	0.45±0.042

There was no significant difference ($p > 0.5$) in haematological parameters at all dose levels compared to the arthritis-only control group. Values are represented as mean $\pm$ SEM, $n = 4$ per group.

The effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on erythrocyte sedimentation rate and haematology parameter in complete Freund’s adjuvant induced arthritis in wistar rats: Figure 1 and Table 3 shows the effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on erythrocyte sedimentation rate and haematology parameters in complete Freund’s adjuvant induced arthritis in Wistar rats. The extract and standard drug reduced (** $p<0.01$) the erythrocyte sedimentary rate compared to arthritis control. There was no significant alteration ($p > 0.05$) in haematological parameters in animals treated with the extract compared to animals in the healthy control group (Table 3).

The effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on biochemical parameters and superoxide dismutase in complete Freund’s adjuvant induced arthritis in Wistar rats: Table 4 shows the effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on biochemical parameters in complete Freund’s adjuvant induced arthritis in Wistar rats. There was no significant alteration ($p > 0.5$) in biochemical parameters in animals treated with the extract compared to animals in the healthy control group. Figure 2 shows the effect of 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* on superoxide dismutase enzyme activity in complete Freund’s adjuvant-induced arthritis in Wistar rats. The plant extract increased (** $p<0.01$, * $p<0.05$) the superoxide Table 4: The effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on biochemical parameters in complete Freund’s

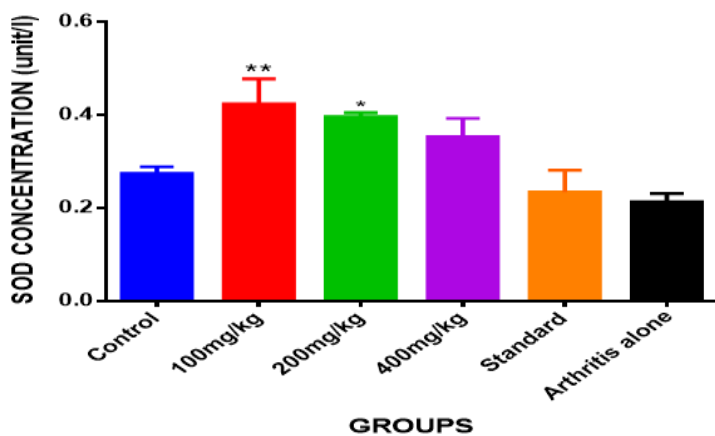
adjuvant induced arthritis in Wistardismutase enzyme activity compared to arthritis control.

Table 4:
The effect of 14-day daily oral administration of 70% methanol extract of *Peperomia pellucida* on biochemical parameters in complete Freund’s adjuvant induced arthritis in wistar rats.

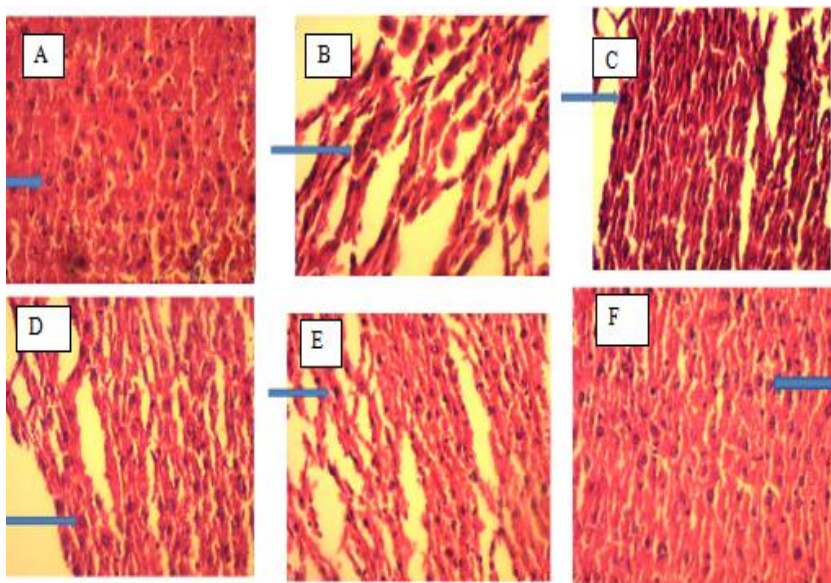
Dose (mg/kg)	AST (UL)	ALT (UL)	ALP (UI/L)	TP (mg/dl)
Control	163.8±6.8	16.0±1.47	15.3±1.48	5.0±0.36
100	207.6±21.4	9.7±3.49	21.6±2.12	3.8±0.48
200	165.4±42.67	13.0±3.19	11.7±0.24	1.4±0.78
400	95.9±21.24	6.9±2.10	15.9±2.03	3.7±1.18
Standard	129.2±7.51	6.4±2.04	15.2±3.79	5.1±0.11
Arthritis alone	115.7±13.09	18.1±8.53	16.4±0.88	6.0±0.57

TP = Total protein
At the 200 mg/kg dose level, there was a significant reduction (** $p<0.01$) in total protein as compared to the arthritis-only control group. Values are represented as mean $\pm$ SEM, $n = 4$ per group.

The histopathology of the liver, kidney, knee joint, and stomach following 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* in complete Freund’s adjuvant induced arthritis in Wistar rats was compared to arthritis control: Plates 1 (slides A - F) 2 (slides G - L), 3 (slides M – Q) and 4 (slide R – X) show the histopathology of the liver, kidney, knee joint, and stomach respectively, following 14-day daily oral administration of a 70% methanol extract of *Peperomia pellucida* in complete Freund’s adjuvant induced arthritis in Wistar rats compared to arthritis control. Animals treated with extract showed significant protection from bone damage compared to the arthritis control group.

**Figure 2:**

The effect of 14 days of daily oral administration of a 70% methanol extract of *Peperonia pellucida* on superoxide dismutase enzyme activity in complete Freund adjuvant induced arthritis in Wistar rats. There was a significant increase (** $p < 0.01$, * $p < 0.05$) in the level of superoxide dismutase enzyme activity in 100 mg/kg and 200 mg/kg as compared to the arthritis-only control group. Values are represented as mean $\pm$ SEM, $n = 4$ per group.

**Plate 1**

A: histological slide of the liver showing normal hepatocytes in the control group (X400).

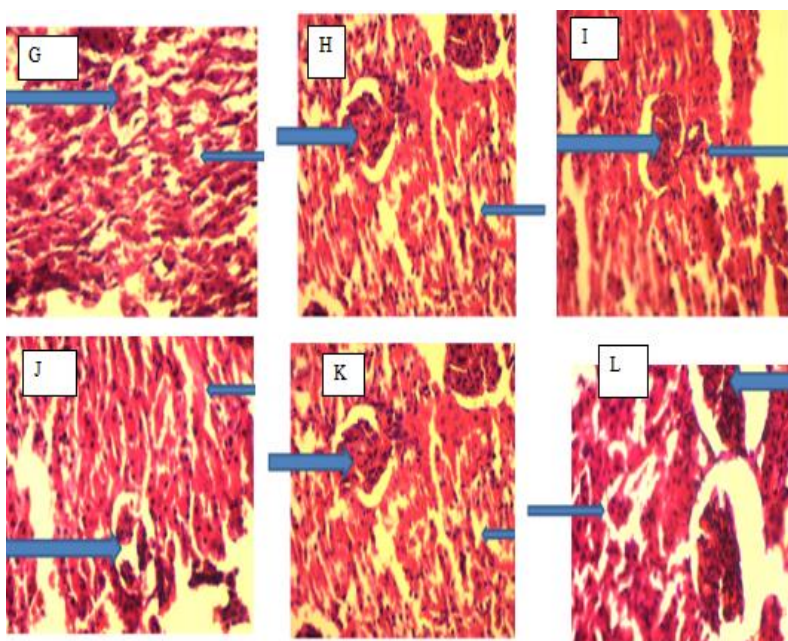
B: histological slide of the liver showing normal hepatocytes in the standard group (X400).

C: histological slide of the liver showing a normal hepatocyte-arthritis control group (X400).

D: Histological slide of liver showing normal hepatocytes in the 100 mg/kg group (X400).

E: Histological slide of liver showing normal hepatocytes in the 200 mg/kg group (X400).

F: histological slide of the liver showing pyknotic hepatocytes in the 400 mg/kg group (X400).

**Plate 2**

G: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the control group (X400).

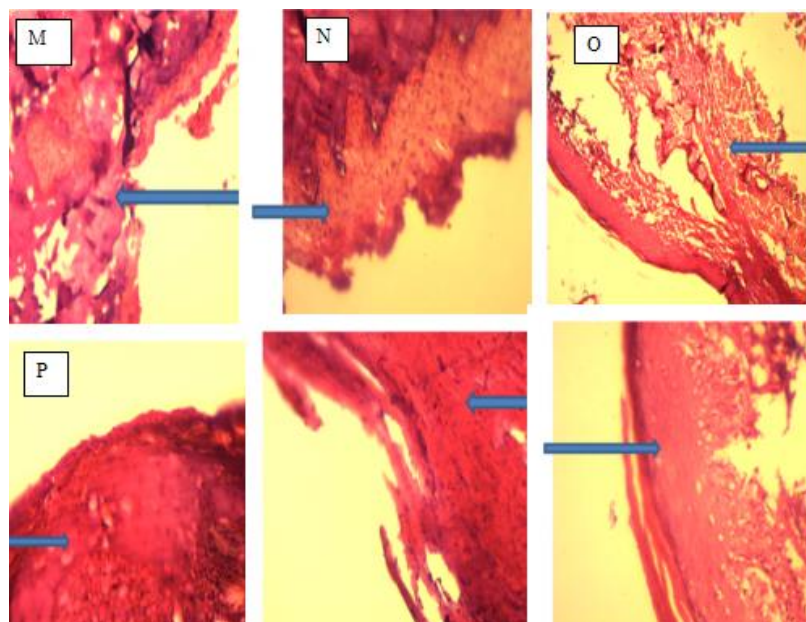
H: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow)—standard group (X400).

I: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow)—arthritis control group (X400).

J: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the 100 mg/kg group (X400).

K: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the 200 mg/kg group (X400).

L: Histological view of the kidney showing normal glomerulus (thick arrow) and normal tubules (thin arrow) in the 400 mg/kg group (X400).

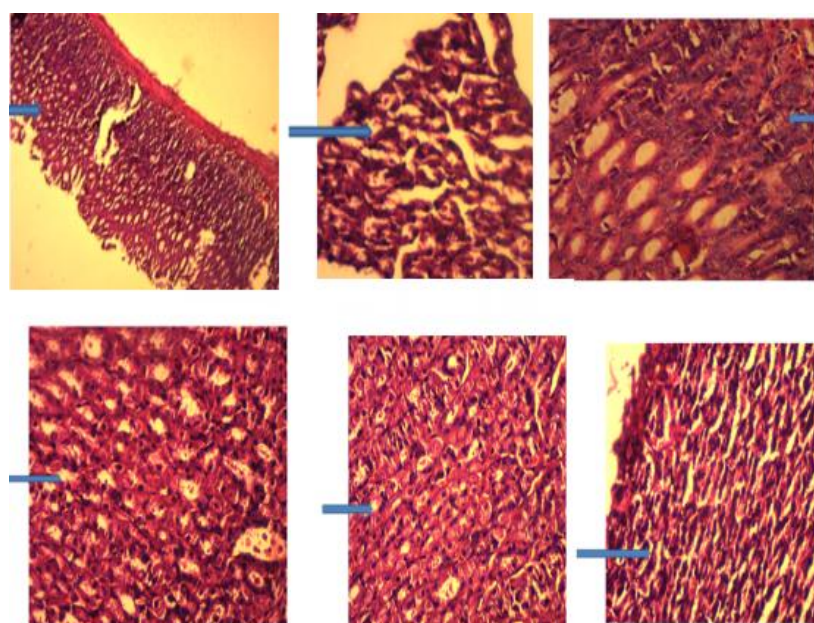
**Plate 3**

M: histological view of the knee joint showing normal fibroconnective tissue in the control group (X100).

N: histological view of knee joint showing normal fibroconnective tissue, standard group (X100). **Plate O:** histological view of knee joint showing fragmentation of connective tissue—arthritis alone group (X100).

P: histological view of the knee joint showing normal fibroconnective tissue in the 100 mg/kg group (X100).

Q: Histological view of knee joint showing normal fibroconnective tissue, 200 mg/kg group (X100).

**Plate 4**

R: Histological view of the knee joint showing normal fibroconnective tissue in the 400 mg/kg group (X100).

S: Histological view of stomach showing normal gastric epithelium - control group (X400).

T: Histological view of the stomach showing normal gastric epithelium, Standard Group (X400).

U: Histological view of the stomach showing necrosis of the gastric epithelium—arthritis alone group (X400).

V: Histological view of the stomach showing necrosis of the gastric epithelium, 100 mg/kg group (X400).

W: Histological view of the stomach showing necrosis of the gastric epithelium, 200 mg/kg group (X400).

X: Histological view of the stomach showing necrosis of the gastric epithelium, 400 mg/kg group (X400).

DISCUSSION

Arthritis is a chronic inflammatory and systemic disease associated with inflammation of the joints, joint damage, synovial inflammation, cartilage damage, and bone damage (Chunxia *et al.*, 2011). The primary symptoms include pain, swelling, and destruction of cartilage and bones, as a result of which permanent disability may occur (Babushetty and Sultanpur, 2012). In most patients, prolonged inflammation rapidly results in bone erosion (Van der Heijde, 1995).

Treatment forms are mainly targeted at eliminating symptoms, stopping the progress of the disease, inhibiting bone destruction, and protecting bone and muscle function (Ichikawa *et al.*, 2012). This study shows the result of 14-day oral administration of a 70% methanol extract of *Peperomia pellucida* in complete Freund's adjuvant induced arthritis in rats. Complete Freund's adjuvant is among the oldest experimental models used for antiarthritis studies (Colpaert *et al.*, 1982). This mimics rheumatoid

arthritis and osteoarthritis in humans (Pathak *et al.*, 2009). In this study, the 70% methanol extract of *Peperomia pellucida* reduced the diameter of inflammation of arthritis in animals treated with the extract when compared with the arthritis control group. The percentage inhibition increased as the dose increased. This means that the anti-arthritis activity of a 70% methanol extract of *Peperomia pellucida* is dose dependent. The percentage inhibitions of the extract at 400 mg/kg (59.04%) and that of the standard drug, methotrexate (56.83%) are comparable (Table 1). Methotrexate is a first-choice drug in the treatment of rheumatoid arthritis. It is a disease modifying antirheumatic drug with cytotoxic and immunosuppressant activity believed to act by blocking adenosine uptake (Chan and Cronstein, 2010). The suppression of inflammation by a 70% methanol extract of *Peperomia pellucida* may be related to this mechanism.

The extract and standard, methotrexate, significantly reduced the erythrocyte sedimentation rate compared to

arthritis control. Erythrocyte sedimentation rate is not specific in diagnosis but a marker related to certain allergies, inflammatory diseases, and infections. It is influenced by the number and size of red blood cells and is involved in the increased formation of endogenous proteins such as fibrinogen and globulins. Clumping of red blood cells is associated with certain inflammatory and autoimmune diseases, and this increases the rate at which cells sink towards the bottom of the test tube. In this study, there was no significant weight change in animals treated with the extract of *Peperomia pellucida*. Weight loss is a potent predictor of health, especially in pathological conditions (Higgins *et al.*, 1993). In this study, the organ-to-body weight ratio was not significantly altered in animals treated with a 70% methanol extract of *Peperomia pellucida* compared to the healthy control group. An increase or decrease in the average organ-to-body weight ratio suggests underlying conditions that may either cause inflammation or shrinkage of internal organs. As a result of the study, the extract of *Peperomia pellucida* supported normal haematological parameters compared to arthritis control.

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are endogenous enzymes that indicate the state and function of the liver, and these values are commonly used to assess the presence of underlying cellular injury (Faqi, 2017). Levels in the blood are increased in certain pathological conditions, including hepatitis, liver damage, and bone and muscle damage (Lala *et al.*, 2021). According to the results of this study, there was no significant alteration in levels of ALP, AST, and ALT in animals treated with 70% methanol *Peperomia pellucida* extract compared to arthritis control.

In this study, the level of endogenous superoxide dismutase enzyme (SOD) was increased in animals treated with an extract of *Peperomia pellucida*. The superoxide dismutase enzyme plays a role in defense against oxidative stress (Younus, 2018). It is an endogenous antioxidant that catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko *et al.*, 2002). It can serve as an anti-inflammatory agent and can also prevent precancerous cell changes (Inal *et al.*, 2001). It has been found to inhibit neutrophil-mediated inflammatory responses (Yasui and Baba, 2006).

In the histopathological study, the knee joints of animals that were given the extract at all doses and standards had healthy fibroconnective tissues. This is contrary to the connective tissue fragmentation obtained in the arthritis control group. Thus, the extract protected the knee joints of the experimental animals from destruction by arthritis induced by complete Freund's adjuvant. There were normal glomeruli and tubules in the kidneys. However, there was necrosis of the gastric epithelium of the stomach in the animals treated with a 70% methanol extract of *Peperomia pellucida* and in the arthritis control group. This suggests that exercising caution is necessary when utilizing an extract of *Peperomia pellucida*.

In conclusion, this study showed that *Peperomia pellucida* possesses anti-arthritis activity. The anti-arthritis activity of *Peperomia pellucida* could be a result of an increase in endogenous superoxide dismutase enzyme activity.

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REFERENCES

- Abere, T. A., Agoreyo, F. O. and Eze, G. I. (2012). Phytochemical, antimicrobial and toxicological evaluation of the leaves of *Peperomia pellucida* (L.) HBK (Piperaceae). *Journal of Pharmaceutical and Applied Sciences*9(3): 1637-1652.
- Arrigoni-Blank, M. F., Oliveira, R. L. B., Mendes, S. S., Silva, P. A., Antonioli, A. R. and Vilar, J. C. (2002). Seed germination, phenology, and antiedematogenic activity of *Peperomia pellucida* (L.) H. B. K. *BMC Pharmacology* 2: 12.
- Athanasίου, K. A., Darling, E. M., Hu, J. C., DuRaine, G. D. and Reddi, A. H. (2013). *Articular Cartilage*. CRC Press p. 105.
- Aziba, P. I., Adedeji, A., Ekor, M. and Adeyemi, O. (2001). Analgesic activity of *Peperomia pellucida* aerial parts in mice. *Fitoterapia*72: 57-58.
- Babushetty, V. and Sultanpur, M. C. (2012). Evaluation of anti-arthritis activity of *Asystasiadalzellianaleaves*. *International Journal of Pharmaceutical Biology Archive*3: 377-382.
- Badami, S., Moorkoth, S. and Suresh B. (2004). *Caesalpinia sappan*, a medicinal and dye yielding plant. *Natural Product Radiance*3: 75-82.
- Britannica, The Editors of Encyclopaedia. (2020). White blood cell. *Encyclopedia Britannica*. Available online at <https://www.britannica.com/science/white-blood-cell>. Accessed 11th July, 2021.
- Camposano, J. E., Torre, D. G. L. T., Laxamana, J. G. and Larcia, L. L. H. (2016). Screening for the anti-angiogenic activity of selected Philippine medicinal plants using chorioallantoic membrane assay. *Mahidol University Journal of Pharmaceutical Sciences*43: 173-182.
- Chan, E. S. and Cronstein, B. N. (2010). Methotrexate--how does it really work?. *Nature reviews. Rheumatology*6(3): 175-178.
- Chitme, R. H. and Patel, P. N. (2009). Antiarthritis activity of *Aristolochiabracteata* extract in experimental animals. *Open Natl Pro J*. 2: 6-15.
- Choudhary, M., Kumar, V., Malhotra, H. and Singhy S. (2015). Medicinal plants with potential anti-arthritis activity. *Journal of Intercultural Ethnopharmacology* 4(2): 147-179.
- Choudhary, M., Singh, S. and Kumar, V. (2020). Anti-arthritis review. *Journal of Intercultural Ethnopharmacology*4(2): 147-179.
- Chukwuma, E. C., Soladoye, M. O. and Feyisola, R. T. (2015). Traditional medicine and the future of medicinal plants in Nigeria. *Journal of Medicinal Plants Studies*3: 23-29.
- Chunxia, C.; Peng, Z.; Huifang, P.; Hanli, R.; Zehua, H. and Jizhou, W. (2011). Extracts of *Arisaema rhizomatum* C.E.C. Fischer attenuate inflammatory response on collagen-induced arthritis in BALB/c mice. *Journal of Ethnopharmacology*. 133: 573-582.
- Colpaert, F. C., Meert, T., Witte, P. and Schmitt, P. (1982). Further evidence validating adjuvant arthritis as an experimental model of chronic pain in the rat. *Life Science*31: 67-75.
- Crofford, L. J. (2013). Use of NSAIDs in treating patients with arthritis. *Arthritis Research and Therapy*15(3): S2.

- Das, N. J., Saikia, S., P., Sarkar, S. and Devi, K. (2006). Medicinal plants of North-Kamrup district of Assam used in primary healthcare system. *Indian Journal of Traditional Knowledge*5: 489-493.
- Deane E. (2021). *Peperomia*. In: Beverage, Edible Raw, Medicinal Plant Uses, Plants Salad, Spice/Seasoning Vegetable. Available online at <http://eattheweeds.com/peperomia/>. Retrieved on 12th May, 2021.
- Florence, N. T., Huguette, S. T. S., Hubert, D. J., Raceline, G. K., Desire, D. D. P. and Pierre, K. (2017). Aqueous extract of *Peperomia pellucida*(L.) HBK accelerated fracture healing in wistar rats. *BMC Complementary and Alternative Medicine*17: 188.
- Frenzkhe, L., Scheiris, E., Pino, G., Symmank, L., Goetghebeur, P., Neinhuis, C. and Samain, M. S. (2015). A revised infrageneric classification of the genus *Peperomia* (Piperaceae). *Taxon*64: 424-444.
- Giboney, P. T. (2005). Mildly elevated liver transaminase levels in the asymptomatic patient. *American Family Physician*. 71(6): 1105-1110.
- Gini, T. G. and Jothi, J. G. (2013). Preliminary phytochemical screening of whole plant extracts of *Peperomia pellucida* (L.) HBK (Piperaceae) and *Marsilea quadrifolia* Linn. (Marsileaceae). *International Journal of Pharmacognosy and Phytochemistry*5: 200-214.
- Hadi, S. and Bremner, J. B. (2001). Initial studies on alkaloids from Lombok medicinal plants. *Molecules*6: 117-129.
- Higgins, M., D'Agostino, R., Kannel, W. and Cobb J. (1993). Benefits and adverse effects of weight loss: observations from the Framingham study. *Annals of Internal Medicine*119: 758-763.
- Humzah, R. U., Odetola, A. A., Erukainure, O. L. and Oyagbemi, A. A. (2012). *Peperomia pellucida* in diets modulates hyperglycemia, oxidative stress and dyslipidemia in diabetic rats. *Journal of Acute Diseases*1: 135-140.
- Ichikawa, N. and Yamanaka, H. (2012). Effect of inflammatory diseases and therapy. *Clinical calcium* 22(2): 215–221.
- Inal, M. E., Kanbak, G. and Sunal, E. (2001). Antioxidant enzyme activities and malondialdehyde levels related to aging. *ClinicaChimica Acta*305: 75–80.
- Kanedi, M., Lande, M. L., Nurcahyani, N., Anggraeni, I. R. and Yulianty, Y. (2017). Hair-growth promoting activity of plant extracts of suruhan(*Peperomia pellucida*) in rabbits. *IOSR Journal of Pharmaceutical and Biological Sciences*12: 18-23.
- Karmen, A., Wroblewski, F. and Ladue, J. S. (1955). "Transaminase activity in human blood". *The Journal of Clinical Investigation*34(1): 126-131.
- Kasper, D. L., Fauci, A. S., Longo, D. L., Braunwald, E., Hauses, S. L. and Jameson, J. L. 16th ed. II. United States of America: Mc-Graw Hill Companies; 2005. *Harrison's Principle of Internal Medicine*.
- Kekuda, P. T. R. and Raghavendra, H. L. (2018). Ethnobotanical uses, phytochemistry and pharmacological activities of *Peperomia pellucida* (L.) kunth (piperaceae)-a review. *International Journal of Pharmacy and Pharmaceutical Sciences* 10(2): 1-8.
- Khan, A., Rahman, M. and Islam, M. S. (2008) Antipyretic activity of *Peperomia pellucida* leaves in rabbit. *Turkish Journal of Biology*32: 37-41.
- Khan, A., Rahman, M. and Islam, M. S. (2010). Isolation and Bioactivity of a Xanthone Glycoside from *Peperomia pellucida*. *Life Science Medicinal Research* 1: 2-12.
- Khan, M. R. and Omoloso, A. D. (2002). Antibacterial activity of *Hygrophilia stricta* and *Peperomia pellucida*. *Fitoterapia*73: 251-254.
- Lala, V., Goyal, A., Bansal P., Minter, D. A. (2021). Liver function tests. In: StatPearls. *StatPearls Publishing*.
- Lans, C. A. (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Journal of Ethnobiology and Ethnomedicine*2: 45.
- Lee, S. W., Sim, K. Y., Wendy, W. and Zulhisyam, A. K. (2016). *Peperomia pellucida* leaf extract as immunostimulator in controlling motile aeromonad septicemia due to *Aeromonas hydrophilia* in red hybrid tilapia, *Oreochromis* spp. *Farming. Veterinary World* 9: 231-234.
- Lipsky, P. E. Rheumatoid arthritis. In: Harrison's Principles of Internal Medicine, (Eds. Kasper, D. L.; Fauci, A. S.; Hauser, S. L.; Longo, D. L. and Jameson, J. L. McGraw Hill, New York: Braunwald E.; 2005. p. 1968.
- March, L., Smith, E. U. R., Hoy, D. G., Cross, M. J., Sanchez-Riera, L., Blyth, F., Buchbinder, R., Vos, T., Woolf, A. D. (2014). Burden of disability due to musculoskeletal (MSK) disorders. *Best Practice and Research Clinical Rheumatology*. 28(3): 353-366.
- Medlineplus.gov. (2020) Alkaline phosphatase. Available online at <https://medlineplus.gov/lab-tests/alkaline-phosphatase/>. Accessed on 15th April, 2021.
- Misra, H. P. and Fridouich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry* 247(10): 3170-3175.
- Mosango, D. M. *Peperomia pellucida* (L.) Kunth. In Prota 11(1): Medicinal Plants / Plantasmédicinales 1; Schmelzer, G. H. and Gurib-Fakim, A. Eds.; PROTA: Wageningen, The Netherlands, 2008.
- Mutee, A. F., Salhimi, S. M., Yam, M. F., Lim, C. P., Abdullah, G. Z. and Ameer, O. Z. (2010). In vivo anti-inflammatory and in vitro antioxidant activities of *Peperomia pellucida*. *International Journal of Pharmacology*6: 686-690.
- Nair, V, O'Neil, C. L and Wang, P. G. "Malondialdehyde", Encyclopedia of Reagents for Organic Synthesis, 2008, John Wiley & Sons, New York.
- Nwokocha, C. R., Owu, D. U., Kinlocke, K., Murray, J., Delgoda, R., and Thaxter, K. (2012). Possible mechanism of action of the hypertensive effect of *Peperomia pellucida* and interactions between human cytochrome P450 enzymes. *Medicinal Aromatic Plants*1: 105.
- Okoh, S. O., Iweriebor, B. C., Okoh, O. O. and Okoh, A. I. (2017). Bioactive constituents, radical scavenging, and antibacterial properties of the leaves and stem essential oils from *Peperomia pellucida* (L.) kunth. *Pharmacognosy Publication*13(3): 392-400.
- Olowa, L. and Demayo, C. G. (2015). Ethnobotanical uses of medicinal plants among the muslimaranaos in Iligan city, Mindanao, Philippines. *Advanced Environmental Biology*9: 204-215.
- Oloyede, G. K., Onocha, P. A. and Olaniran, B. B. (2011). Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomia pellucida* from Nigeria. *Advances in Environmental Biology*.
- Pathan, P., Nimish, I., and Bhalodia, Y. (2008). Curative effect of albizialebeck methanolic extract against adjuvant arthritis-with special reference to bone erosion. *International Journal of Pharmaceutical Sciences and Drug Research*1(3): 110-121.
- Petrovska, B. B. (2012). Historical review of medicinal plants usage. *Pharmacognosy Reviews*6(11): 1-5.

- Ragasa, C. Y., Dumato, M. and Rideout, J. A. (1998). Antifungal compounds from *Peperomia pellucida*. *ACGC Chemical Research Community* 7: 54–61.
- Santos, M. R. A., Lima, M. R. and Oliveira, C. L. L. G. (2014). Medicinal plants used in Rondônia, Western Amazon, Brazil. *Revista Brasileira de Plantas Mediciniais* 1: 707-720.
- Sonibare, M. A., Moody J. O. and Adesanya, E. O. (2009). Use of medicinal plants for the treatment of measles in Nigeria. *Journal of Ethnopharmacology* 122: 268-272.
- Tandon, V. and Gupta, R. K. (2004). Histomorphological changes induced by *Vitex negundo* in albino rats. *Indian Journal of Pharmacology* 36: 176–177.
- Uwaya, D. O. MacDonald, I. Ikononwan, J. O. and Gabriel, B. O. (2022). Anti-arthritis potential of aqueous leaf extract of *Combretum platypterum* (Welw) Hutch & Dalziel (Combretaceae) on rats. *Journal of Current Biomedical Research*, 2(5): 440 - 455
- Van der Hejide, D. M. (1995). Joint erosions and patients with early rheumatoid arthritis. *The British Journal of Rheumatology* 34(2): 74-78.
- Vispute, S. and Khopadae A. (2011). *Glycyrrhiza glabra*, L. Klitaka: A review. *International Journal of Pharmacology and Biological Sciences* 2: 42–51.
- Wambugu, S. N., Mathiu, P. M., Gakuya, D. W., Kanui, T. I., Kabasa, J. D. and Kiama, S. G. (2011). Medicinal plants used in the management of chronic joint pains in Machakos and Makueni countries, Kenya. *Journal of Ethnopharmacology* 137(201): 945-955.
- Xu, S., Li, N., Ning, M. M., Zhou, C. H., Yang, Q. R. and Wang, M. W. (2006). Bioactive compounds from *Peperomia pellucida*. *Journal of Natural Products* 69: 247–250
- Yasui, K. and Baba, A. (2006) Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. *Journal of Inflammation Research* 55: 359–363.
- Zelko, I. N., Mariani, T. J. and Folz, R. J. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radical Biology and Medicine* 33(3): 337-349.
- Zubair, K. L., Samiya, J. J., Jalal, U. and Mostafizur, R. (2015). In vitro investigation of antidiarrhoeal, antimicrobial and thrombolytic activities of aerial parts of *Peperomia pellucida*. *Pharmacology Online* 3: 5-13.

Full length Research Article

Effect of *Musa paradisiaca* Stem Juice on Acute Status Epilepticus, Hippocampal Histology and Behaviour in Rats with Pentylenetetrazole-Induced Seizures

Bassey I.F.^a, Eru E.M.*^a, Udoaffah G.U.^a, Nnenna W.A.^a,
Ajaba M.O.^b, Anani S.E.^a, Umoh N.M.^a, Ekanem A.E.^a, Okpabi F.^b, Ushie A.^c

^aDepartment of Anatomical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.

^bDepartment of Science Laboratory Technology, University of Calabar, Calabar, Cross River State, Nigeria.

^cFaculty of Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria.

Summary: Status epilepticus, histology, and neurobehavioral parameters of pentylenetetrazole-induced epileptic wistar rats were being investigated following administration of *Musa paradisiaca* (MP) stem juice. A seizure was induced sixty minutes after the administration of graded doses of *Musa paradisiaca* stem juice and diazepam, an anticonvulsant drug. A neurobehavioral test was carried out after pentylenetetrazole (PTZ) induction. The animals were arbitrarily given five groups, namely: A, B, C, D, and E. Group A animals functioned as the normal control, given rat pellets and distilled water. Group B served as the PTZ control, and the animals were administered 65mg/kg body weight of PTZ intraperitoneally. Group C served as the PTZ + Diazepam (DZP) treated group, and the animals were administered orally 4 mg/kg body weight of diazepam 60 minutes before administration of PTZ intraperitoneally. Group D and E served as the PTZ + MP [Low dose and High dose] pre-treated groups, given 2500 mg/kg and 5000 mg/kg body weight of MP stem juice, respectively, for 7 days orally before administration of PTZ intraperitoneally and 1 hour post treatment with MP stem juice. Significant antiepileptic effects on status epilepticus were observed in MP stem juice (2500mg/kg and 5000mg/kg) and the Diazepam group of animals. These antiepileptic effects were clearly seen in the delay recorded in latency of seizure and latency of tonic-clonic seizure, a reduced frequency of myoclonic jerks and a 100% mortality index rate recorded in the MP stem juice pre-treated animals. Histological sections of the hippocampus pre-treated with *Musa paradisiaca* stem juice and diazepam revealed significant differences in the structural integrity when compared with the epileptic control. The novel object recognition task test carried out showed a positive score for long-term memory as attained by animals in Group D and E when compared to Group B animals that had a negative score for long-term memory. In this study, the administration of *Musa paradisiaca* stem juice limited the extent of status epilepticus, reduced cytoarchitectural damage caused by epilepsy, and further enhanced long-term recognition memory in the MP stem juice pre-treated epileptic rats.

Keywords: Pentylenetetrazole, *Musa paradisiaca*, Status epilepticus, Neurobehavioral

*Authors for correspondence: erumba@unical.edu.ng, Tel: +2347065560843

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INTRODUCTION

The brain is considered a master organ due to its ability to regulate the activity of the entire human system. It is composed of a number of subcomponents that are responsible for organizing reflexes and other behavioural responses, as well as for cognition, learning, memory, planning, and the execution of voluntary movements (Singh, 2004). Under the medial temporal lobe of the cerebrum, one on each side of the brain, lies the hippocampus. The hippocampus, along with its adjacent temporal and parietal lobe structures, constitutes the hippocampal formation (Guyton and Hall, 2000). The hippocampal formation is involved in the creation of new historical and truthful memories, and it also serves as a "memory gateway"

through which new memories must pass before being permanently stored in the brain (Serra *et al.*, 2020). Anterograde amnesia will result from damage to the hippocampus, which is the inability to form and retain new memories. According to Anand and Dhikav (Anand and Dhikav, 2012), the hippocampus is a common focus site in epilepsy and may be harmed by chronic seizures. The brain's high complexity makes it susceptible to damage. According to a 2015 report by the National Institute of Neurological Disorders and Stroke, difficult births, poor nutrition, and exposure to infectious diseases and toxins can cause or aggravate neuropsychiatric disorders. Hundreds of millions of people worldwide are affected by neurological disorders. Every year, more than 6 million people die as a result of a

stroke, with low- and middle-income countries accounting for more than 80% of these deaths according to a review by Leone *et al.*, (2021). In this review, about 60 million people have epilepsy worldwide.

Epilepsy is derived from the Greek word "epilepsia," which is composed of two words: "epi" meaning "upon" and "lepsi" meaning seizures (Harper, 2001). This neurologic disorder disrupts the transmission of electrical signals within the brain and it is marked by a persistent propensity to generate epileptic seizures, as well as neurobiological, cognitive, psychological, and social consequences of this condition (Fische *et al.*, 2005). There is a difference between seizures and epilepsy [also known as a "seizure disorder"]. A seizure is a temporary incidence of indications caused by an abnormal increase or synchronous neuronal activity in the brain (Bazil *et al.*, 2019). All human cerebral cortices are capable of producing seizures if sufficiently stimulated. According to research, approximately 10% of individuals will have one at some point in their lives (Epileptic Foundation, 2010). The term "epilepsy" refers to a condition in which a person has an abnormally elevated likelihood to have seizures, such that they are susceptible to having them on a regular basis during normal life (World Health Organization, 2019). Using electroencephalograms, which detect abnormal neuronal signals in the brain, the origin of seizures can be determined (Fischer *et al.*, 2014). These seizures occur occasionally or on regular basis, are most prevalent in adults, and have been hypothesized to cause mental issues, such as high rates of depression and anxiety. Therefore, epilepsy has a profound impact on the entire central nervous system. Stress, sleep deprivation or fatigue, insufficient food intake, alcohol or drug abuse, and failure to take prescribed anticonvulsant medications may increase the risk of seizures in susceptible individuals (Diaz-Negrillo, 2013). Epilepsy is considered extremely dangerous, especially if the condition status epilepticus (SE) develops. Status epilepticus is defined as epileptic activity that persists for 30 minutes or longer. According to the International League against Epilepsy Task Force on Classification of Status Epilepticus, SE conceptually refers to "a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms which lead to abnormally prolonged seizures" (Trinka *et al.*, 2013). Seizures can manifest as either prolonged or recurrent attacks without periods of recovery in between. This condition was estimated to affect over 100,000 Americans annually and result in approximately 50,000 deaths (World Health Organization, 2009). 31% to 43% of patients with SE do not respond to standard medications (Mayer *et al.*, 2002). Without immediate care, this condition can result in permanent brain damage or death. In an early foundational work on primate models, Meldrum and Horton (1973) and Meldrum *et al.*, (1973) described associated neuronal loss in SE. Even in the absence of hypoxia, acidosis, hypoglycaemia, and other confounding factors, programmed necrosis and apoptosis may mediate cell death according to (Niquet *et al.*, 2003).

In mice, rats, cats, and primates, pentylenetetrazole (PTZ), a tetrazol derivative, induces convulsions. It does so apparently by blocking GABA-mediated inhibition through an action at the GABA receptor (Olsen, 2001; Klioueva *et al.*, 2001). For research into brain excitability and the

development of antiepileptic medicines, PTZ injection is a popular behavioural technique (Loscher, 2002; Pitkanen *et al.*, 2015). PTZ is a model drug for generalized seizures, according to the behavioral and electroencephalogram (EEG) manifestations of rodent seizures brought on by the drug. When used to cause recurring seizures, PTZ has been used as a model for generalized tonic-clonic seizures. PTZ can be given subcutaneously, intravenously, or intraperitoneally. The amount and method of administration impact the latency to seizure onset and behavioural symptoms; typically, a single systemic administration is sufficient to elicit seizures. PTZ can result in status epilepticus when given in large doses (Holmes and Ben-Ani, 1998). Young rats can also be repeatedly given PTZ in the kindling model of epilepsy (Erkeç and Arihan, 2015; Aiyeloja and Bello, 2006).

The plantain, *Musa paradisiaca*, belongs to the Musaceae family and is an herbaceous plant that can grow up to 9 meters long. It has a strong pseudo-tree-like pseudo-stem and a crown of large, elongated, oval, dark-green leaves with a prominent midrib that can measure up to 365 cm in length and 61 cm in width. More than 70 million people in sub-Saharan Africa rely on it as a staple meal, and it is widely produced in the tropics.

It is known as Ogede (Yoruba), Abrika (Igbo), Okamuyaba (Hausa), and Ukom (Efik/Ibibio) in Nigeria (Ajaba, 2016; Onyenekwe *et al.*, 2013). According to Onyenekwe *et al.*, (2012), plantain juice (pseudo stem) contains alkaloids, saponins, flavonoids, glycosides, anthraquinones, phlobatannins, and tannins. It has been demonstrated that flavonoids prevent the prompting, promotion, and progression of tumors due to their anti-oxidant properties (Citraro *et al.*, 2016). Oxidative stress may be involved in the initiation and progression of epileptogenesis as a result of excessive free-radical production. A recent report by Citraro *et al.*, (2016) on the anticonvulsant effect of flavonoid-rich extract from *Citrus sinensis* (sweet orange) in certain experimental models of epilepsy provides additional support for the neurorehabilitative effect of this active ingredient (flavonoid). These phytochemicals provide *Musa paradisiaca*'s juice its synergistic therapeutic qualities, which explains why it is used to treat a wide range of illnesses (Fischer *et al.*, 2019; Kim *et al.*, 2012; Agbo *et al.*, 2015; Agbo *et al.*, 2019; University of Florida Herbarium, 2022).

In light of the foregoing and other studies on the anti-oxidative effects of flavonoid-rich extracts, this study assessed the antiepileptic activity, learning, and memory effects of *Musa paradisiaca* stem juice in a PTZ-induced model of epilepsy

MATERIALS AND METHODS

Experimental animals: Thirty male albino wistar rats weighing between 110-160g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar. The animals were allowed one week of acclimatization and housed in cages under room temperature (25±20C), relative humidity (50±5%) and a 12 hour light/dark cycle in the animal house of the Faculty of Basic Medical Sciences. During the whole experiment, the animals could eat rat food and drink tap water whenever they wanted.

Sample collection and preparation: Fresh plantain pseudo stems used for this experiment were obtained from the plantation of Mrs. Kinini Frank in Ikot Nkebre, 8 miles from Calabar Municipality, Cross River State. The stems were authenticated at the Department of Botany, University of Calabar, Nigeria and the voucher specimen was placed in the Department's herbarium with voucher number HERB/BOT/UCC/ 145. Plantain stems were cleaned of dirt by washing. To extract the juice, they were then cut into slices and crushed with a mortar and pestle. At least 600ml of the juice were extracted and stored for further use right away at 4°C day and night. This method is according to University of Florida Herbarium (UFH) (2022)

Acute toxicity test: Oral acute toxicity of *Musa paradisiaca* juice was determined in rats as described by Lorke (1983). The maximum tolerated dose in the current study was determined to be larger than 5000 mg/kg after it was determined that *Musa paradisiaca* juice has no drug-related toxicity.

Chemicals used: Both the chemicals (PTZ) and the drugs (Diazepam) were obtained commercially from Bez Pharmacy, Calabar, Cross River State, and were of analytical grade. Thus, the chemicals are ultra-pure and are ideal for providing consistent results.

Drug preparation: PTZ was freshly prepared to the desired concentration of 13mg/ml (195mg of PTZ dissolved in 15ml of normal saline). The drug will be given at a dose of 65mg/kg bodyweight by taking volumes of the stock corresponding to the dose for each rat.

Experimental design: The design consisted of 30 male Wistar rats divided into 5 groups (A-E) of 6 animals each. The groups were a normal control, an epileptic, and the 3 epileptic treated groups. In this study, diazepam was used as a reference drug to check anti-convulsing activity in PTZ induced epilepsy. The doses used were based on the LD50 determined for the juice, which was greater than 5000mg/kg. Group A animals served as the normal control and were administered rat pellets and distilled water for 14 days. Group B animals that served as the epileptic control received 65mg/kg body weight of pentylenetetrazole once. Group C animals were treated with 4mg/kg body weight of Diazepam before and after epilepsy induction. Group D was the *Musa paradisiaca* juice group and received 2500mg/kg body weight of *Musa paradisiaca* stem juice daily for 7 days, while group E animals received 5000mg/kg body weight of *Musa paradisiaca* stem juice daily for 7 days.

The drugs (PTZ and Diazepam) and extracts were administered by intraperitoneal (I.P) and oral routes, respectively. Seven (7) days of pre-treatment with the juice of MP, one-hour pre/post treatment with diazepam, and a one-hour post treatment with 65mg/kg of PTZ, were given to the animals, and the animals were observed over a period of thirty minutes after. During this period of observation, the onset of seizures (time it takes for seizures to begin), latency to seizure, frequency of seizures, and mortality rate were noted. From the data obtained, quantal protection (number of animals alive in each group divided by the total number of animals in the group), percentage protection (percentage

of animals alive) and percentage mortality (percentage of animals dead) were calculated.

Ethical approval: Approval was obtained for all experimental procedures from the Faculty Animal Research Ethics Committee of the Faculty of Allied Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria with an Approval number: 086ANA2321

Collection of tissue samples for analysis: At the conclusion of the 14-day experiment, the animals were sacrificed and perfused using chloroform vapour and dissected. Their brains were surgically excised, the hippocampus localized and fixed in formal saline preparation for histological analyses.

Histological evaluation: Formal-saline fixed tissue sections of the hippocampus were processed for histopathological examination through careful histological procedures in a specific order that included dehydration, clearing, impregnation, embedding, sectioning, slide preparation, staining, and microscopy. The tissue sections were stained using haematoxylin and eosin (H&E) as described by Bancroft and Stephens (1982). Images were captured with a light microscope at a magnification of X400.

Novel Object recognition task: The object recognition task is conducted in an open field (OF) box (38 x 38 cm), which is also used to assess exploratory behaviour. All animal testing were conducted under diffuse lighting conditions via a 60-Watt white light bulb. A video camera suspended above the centre of the open field records all behaviours for later analysis. Behaviour were scored live using a video camera-based computer tracking system (Lime Light, Actimetrics). The computerized event-recorder program Hindsight for MS-DOS version 1.5 can also be used to record behaviour.

Statistical analysis: Data were presented as mean $\pm$ standard error of mean. Data were computed and analyzed using one way ANOVA to compare group means of antiepileptic activity and neurobehavioral test. The data were analyzed with the help of a statistical package, SPSS version 22.0 for Windows, considered significant at $p < 0.05$.

RESULTS

Latency of seizure: The latency of seizure recorded for groups C, D and E was 350.86 ± 64.38 , 373.00 ± 60.25 , and 299.18 ± 56.28 sec respectively. These values were significantly higher ($P < 0.05$) compared to group B, which recorded 66.69 ± 3.24 (Figure 1).

Latency of tonic-clonic seizure: Treated groups (C, D, and E) exhibited significantly higher values of 589.88 ± 190.76 , 618.39 ± 56.66 and 1655.79 ± 126.49 sec, respectively, to delay the initiation of tonic-clonic seizure in the animals as compared to PTZ control group B, which only delayed it for about 132.37 ± 7.47 sec (Figure 2). In group D (Pentylenetetrazole + *Musa paradisiaca* [High dose]), this effect on how long it took for a tonic-clonic seizure to start was more noticeable.

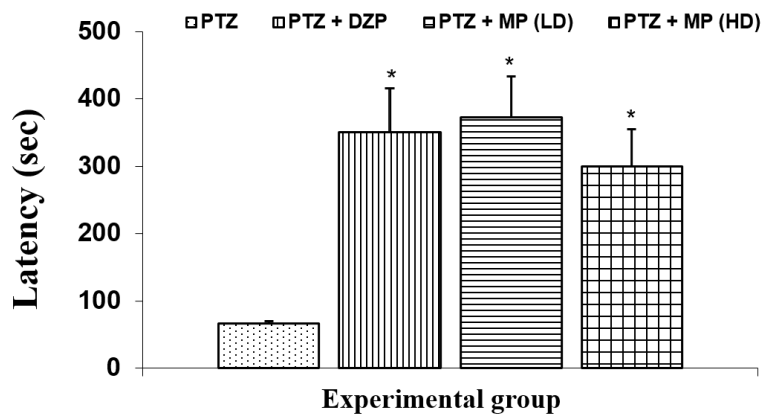


Figure 1:
Comparison of latency of seizure in the different experimental groups.
Values are expressed as mean $\pm$ SEM, $n = 5$. * = $p < 0.05$ vs PTZ

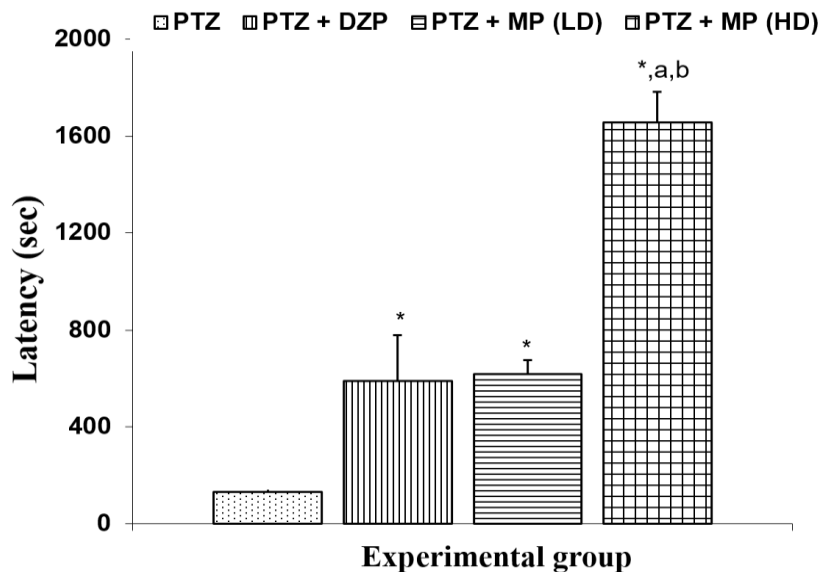


Figure 2:
Comparison of latency of tonic clonic seizure in the different experimental groups.
Values are expressed as mean $\pm$ SEM, $n = 5$.
* = $p < 0.05$ vs PTZ; a = $p < 0.05$ vs PTZ + DZP
b = $p < 0.05$ vs PTZ + IM (LD)

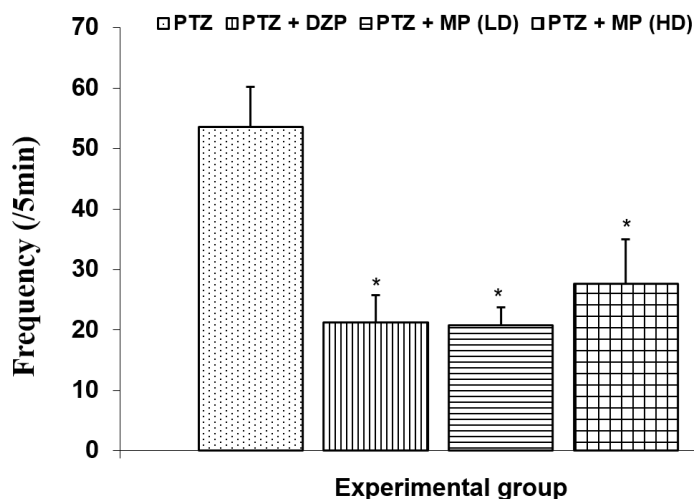


Figure 3:
Comparison of frequency of jerks in the different experimental groups.
Values are expressed as mean $\pm$ SEM, $n = 5$. * = $p < 0.05$ vs PTZ

The Frequency of Myoclonic Body Jerks: The frequency of myoclonic jerks was also observed and the result showed that PTZ control rats (group B) exhibited jerks for 53.60 ± 6.62 minutes in contrast to the treated rats (group C, D and E) with significantly lower duration for their jerks, which were recorded as 21.25 ± 4.49 , 20.80 ± 2.94 , and 27.60 ± 7.41 minutes. (Figure 3)

Mortality index rate: Seizure phases developed in these animals amongst experimental groups B, C, D, and E were ear and facial twitching, myoclonic body jerks, clonic

forelimb convulsions, generalized clonic convulsions, turning onto one side position, and generalized clonic tonic convulsions (or death within 30 minutes). These seizure phases will sequentially result in mortality cases recorded amongst the experimental population. A 50% mortality index rate was recorded in group B (PTZ), a 16.67% mortality index rate for group C (PTZ + DZP), a 0% mortality index rate and 100% protection rate for group D (PTZ + MP [Low dose]) and group E (PTZ + MP [High dose]) (Table 1).

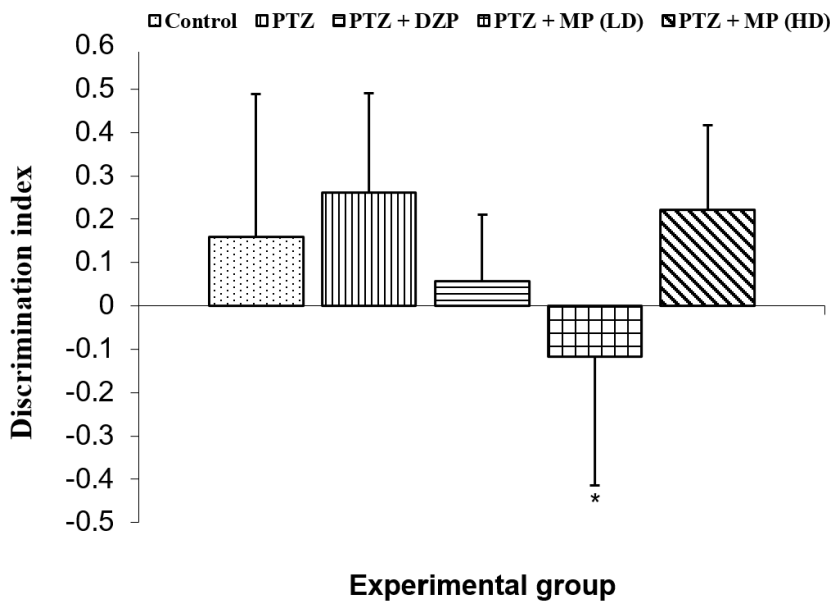


Figure 4:
Comparison of discrimination index for short term memory during Novel object recognition task in the different experimental groups. Values are expressed as mean +SEM, n =5

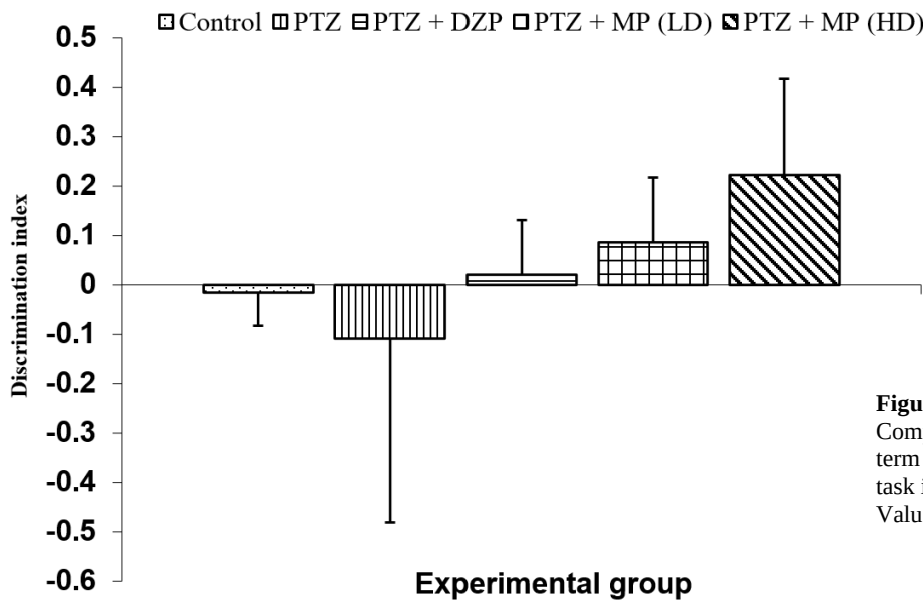


Figure 5:
Comparison of discrimination index for long term memory during Novel object recognition task in the different experimental groups. Values are expressed as mean +SEM, n =5

Table 1:
Effect of *Musa paradisiaca* stem juice on PTZ-induced seizure

Group	Treatment / Dose	No of rats	Quantal Protection	Percentage Protection %	Percentage Mortality %
A	Normal control (Distilled water)	6	-	-	-
B	PTZ (65mg/kg.b.w)	6	3/6	50	50
C	PTZ + DZP (4mg/kg.b.w)	6	5/6	83.33	16.67
D	PTZ+MP Juice (2500mg/kg.b.w)	6	6/6	100	0.00
E	PTZ+MP Juice (5000mg/kg.b.w)	6	6/6	100	0.00

Histological findings: The hippocampus of group B (PTZ control) animals induced with epilepsy and not treated showed a distorted architecture, a reduction in neuronal cell population, pyknosis, and chromatolysis (Plate 1B). In the *Musa paradisiaca* treated groups (Plate 1D and 1E), there was a marked reduction in cellular damage, which also had similar features to group C (Plate 1C). Group E (Plate 1E) showed realignment of cellular layers, with the molecular layer, polymorphic cell layers, and pyramidal layer having

densely populated neuronal cells with prominent nuclei, scanty glia cells, and blood vessels. Sections from the normal control group showed the hippocampus consisting of an outer molecular layer and an intermediate pyramidal layer. The pyramidal cell layer consists of densely populated and closely packed pyramidal cells of various sizes with prominent nuclei. Interspersed in these two layers were microglia cells (Plate 1A). This is in contrast to animals that were administered 65mg/kg body weight of PTZ only.

Sections from this group showed a partially distorted architecture with indistinct layers that appeared to consist of sparsely populated neuronal cell bodies with an obvious shrinkage of the cell body, pyknosis, and intense chromatolysis. The pyramidal cell layer appears scanty and degenerated pyramidal cells are seen (Plate 1B). Observations made on the treated groups began with animals that were given 4 mg/kg body weight of diazepam before and after epileptogenesis. The sections showed the hippocampus with distinct cellular layers. The pyramidal cell layer is clearly visible and appears to consist of a scanty population of pyramidal cells with prominent oval to ovoid nuclei. Intervening blood vessels were also seen between the molecular and pyramidal cell layers (Plate 3). Similar changes were also noted in an animal group administered 2500mg/kg body weight of *Musa paradisiaca* stem juice extract. The section of the hippocampus showed realignment of the different cell layers, which are now distinct. The pyramidal cell layer appeared filled with neuronal cells which had round to ovoid nuclei. There is also the presence of microglia cells (Plate 4). Sections of the hippocampus in animals treated with 4000 mg/kg body weight of *Musa paradisiaca* stem juice showed distinct layers having densely populated neuronal cells with prominent nuclei. They were microglia cells and intervening blood vessels between molecular and pyramidal layers (Plate 5). These changes can be compared to those in the normal control group of animals.

DISCUSSION

In this study, the juice (2500mg/kg and 5000mg/kg b.w) from the stem of *Musa paradisiaca* exhibited significant antiepileptic activities in the PTZ-induced model of epilepsy. This was observable as the juice showed a significant effect on the recorded seizure parameters, analyzed histological sections, and neurobehavioural assessment. Seizure parameters recorded were latency of seizure, latency of tonic clonic seizure, frequency of myoclonic jerks, and mortality rate analysis.

Goldberg and Coulter (2013) alludes that latency of seizure is the time between the start of epilepsy (epileptogenesis) and the first seizure. Tonic-clonic seizures, on the other hand, are distinguished by clonic or myoclonic movements that progress to tonic muscle extension of the limb and trunk muscles followed by clonus, and they can result in significant morbidity and mortality, according to Hammond (2016). The juice from *Musa paradisiaca* stem recorded a significant delay in the action of PTZ in the treated groups as compared to the PTZ control rats. This effect was evidenced by a significant delay in the latency of seizure and tonic clonic seizure among the juice treated groups D and E, as compared to the PTZ control group (Figure 1 and 2). These results are in similitude with reports from Reddy *et al.*, (2018) on the effect of *Musa sapientum* stem extract on acute and chronic experimental models of epilepsy.

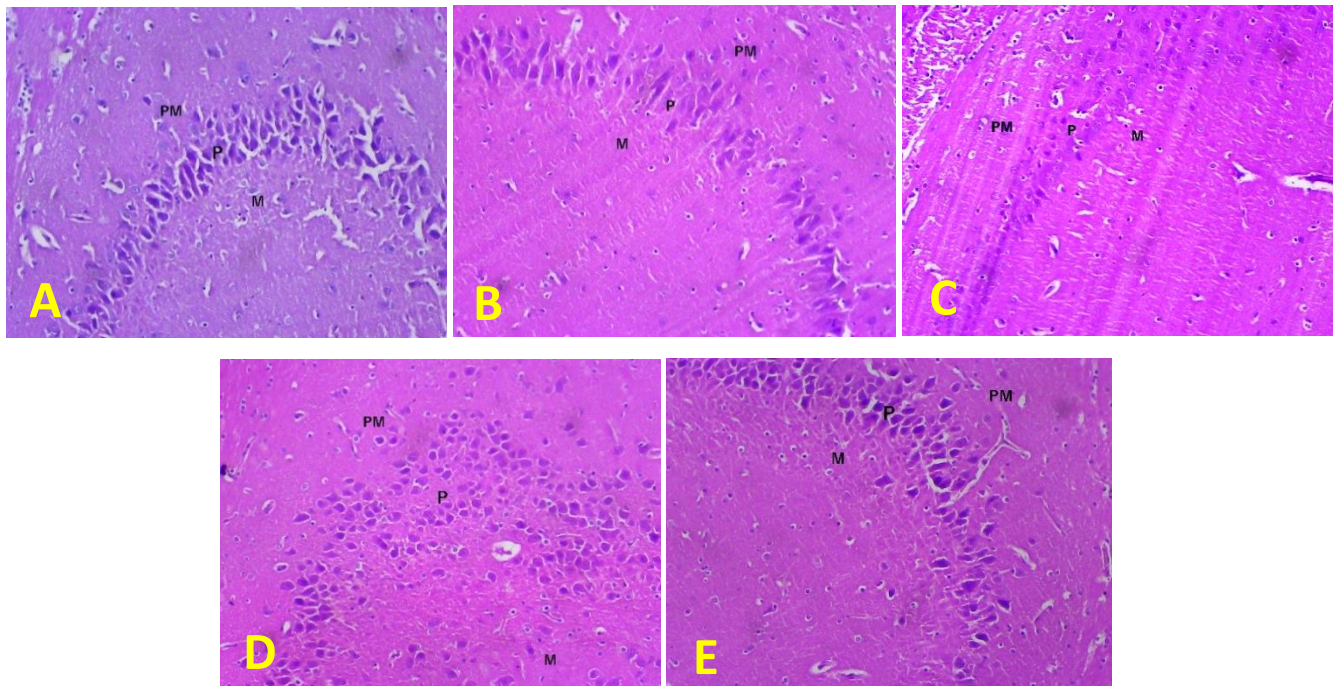


Plate 1 (A-E)

1A: Section of hippocampus from group A (normal control) showing the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) with densely populated and closely packed pyramidal neurons (P).

1B: Section of hippocampus from group B (PTZ control) showing indistinct and partially distorted cellular architecture; the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) with sparsely distributed pyramidal neurons (P).

1C: Section of hippocampus from group C (PTZ + DZP) showing distinct cellular layers; showing molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P). The pyramidal cell layer is clearly visible and appeared to consist of scanty population of pyramidal cells.

1D: Section of hippocampus from group D (PTZ + MP [LDJ]) showed realignment of the different cell layers which are now distinct; the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) with numerous pyramidal neurons.

1E: Section of hippocampus from group E (PTZ + MP [HDJ]) showed distinct layers; the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) having densely populated neuronal cells with prominent nuclei.

Ameliorative potential of Musa paradisiaca juice on PTz-induced epileptic rats

In line with this research, there was a significant increase in the latency to the onset of myoclonic jerks and the duration of clonic convulsions following PTZ administration.

According to Shibasaki and Hallet (2005), myoclonus is a movement disorder that presents itself with sudden, brief, shock-like jerks. Kojovic *et al.*, (2011) reviewed that myoclonus may be classified as physiological, essential, epileptic, symptomatic, or psychogenic based on aetiology. In the present study, as expected, the PTZ control group of animals initially produced myoclonic jerks, which subsequently became sustained and led to a generalized tonic-clonic seizure. In addition, it was observed that the frequency of myoclonic jerks in the treated groups (C, D, and E) decreased much more rapidly than in the PTZ control rats (group B), which exhibited a higher frequency of jerks.

The MP stem juice also offered 100% quantal protection to the treated animals in groups D and E, thereby significantly reducing seizure severity posed by the chemoconvulsant drug (PTZ) without any record of mortality in this group. This is in contrast to the PTZ-induced group of animals, where half of the population died due to seizure severity (Table 1). In line with this result, Massey *et al.*, (2014) reported that severity of seizures leads to sudden unexplained death in epileptic patients (SUDEP) whose mechanisms remain unknown, with both cardiac and pulmonary derangement having been hypothesized. Stachowicz *et al.*, (2008) also emphasized that seizure severity leads to a convulsive syndrome, which is a multi-causal phenomenon characterized by the decrease or absence of neuronal inhibition usually mediated by Gamma amino butyric acid. His reports still follow that PTZ antagonizes GABAergic receptors, leading to a decrease in GABAergic tone, which is associated with an increase in neuronal excitability that favours the hyper synchronization of neuronal electrical activity and the appearance of convulsive episodes. This will in turn trigger numerous pathologies which will result in increased mortality. The MP stem juice administered to the treated animals acted to reduce this seizure severity by neutralizing PTZ levels in the blood of these treated animals, thereby minimizing its dose-dependent action on GABAergic receptors and avoiding the death that would have ensued. The neutralizing effect of MP stem juice has also been reported by Borges *et al.*, (2005). Positive responses of extract-treated animals during this phase of seizure evaluation may be attributable to the capacity of MP stem juice to neutralize specific chemical components. Furthermore, these aforementioned antiepileptic activities of the MP stem juice may be due to the presence of phytochemicals in the juice of the *Musa paradisiaca* plant, as well documented by Akpuaka and Ezem (2011) and Akpabio *et al.*, (2012)]. In particular, Ostróžka-Cieślak (2002) reported that antioxidants are essential to protect the human organism. This will support our findings because in this study, the high concentrations of flavonoids in the MP stem juice administered to the animals in group D (PTZ + MP) had the greatest impact, blocking the toxic effects of PTZ on neuronal excitability and preventing convulsion-induced crises.

Epilepsy is typically associated with prominent cell loss and altered cytoarchitecture of the brain, specifically the hippocampus, according to research (Arellano *et al.*, 2004). Additionally, numerous deformities, such as loss of

plasticity, are commonly observed in affected neurons. The extent of metabolic workup is governed by the type of seizure and syndrome (Pearl, 2004). An imbalance between generated reactive metabolites, also known as reactive oxygen species (ROS), and the body's antioxidant system is known to cause oxidative stress. However, if the imbalance persists, it can cause cell damage and eventually disease. Seizures and seizure-induced neuronal damage are facilitated in part by free radical damage, which has been identified as a key factor (Shin *et al.*, 2011; Frantseva *et al.*, 2000). In sequence to this finding, in the current study, the hippocampus of PTZ control animals induced with epilepsy and not treated showed a distorted architecture, a reduction in neuronal cell population, pyknosis, and chromatolysis. This corresponds to reports from Sutula *et al.*, (2002) that prolonged seizure characteristics which may progress to tonic-clonic convulsive seizures are more likely to lead to neuronal loss. On the other hand, Olajuyigbe and Afolayan (2011) demonstrated in their study that phenols and flavonoids, which are excellent antioxidants resident in MP stem juice, would greatly mop up free circulating radicals that may result in neuronal damage, and thus in the *Musa paradisiaca* treated groups (D and E), there was a marked reduction in cellular damage, which also had similar features to group C (PTZ + DZP). Broadbent *et al.*, (2010) reported that the hippocampus is important for object recognition memory, and if there are lesions on this structure, moderate and reliable anterograde memory impairment will occur. While Eru *et al.*, (2022; 2021a; 2021b; 2020a, 2020b) linked hippocampal cells death with learning and memory enhancement in cognitive dysfunction rats induced with scopolamine hydrobromide post treatment with *Telfairia occidentalis* seeds and *Talinum triangulare* leaves aqueous extract, Anani *et al.*, (2020) and Udoh *et al.*, (2020) reported the neuroprotective effect of *Averrhoa carambola* aqueous fruit extract on the hippocampus and *Cytrus esculentus* reversed radiation-induced damage testicles in rats respectively. In light of these reports, the anatomico-functional relationship of the hippocampus was meticulously examined in this study. The novel object recognition test was used to conduct a neurobehavioral test for learning and memory. According to Lueptow (2017), the novel object recognition task is an efficient and adaptable method for studying learning and memory in rats and is appropriate for the detection of neuropsychological changes following pharmacological, biological, or genetic manipulations. Oliveira *et al.*, [(2010) added that during a NOR task, memory is consolidated and spatial or contextual characteristics of objects can be relocated in different parts of the brain. The discrimination index is the most common memory measurement for the novel object task (Denninger *et al.*, 2018). A comparison of the discrimination index for short-term memory revealed that treated animals in the MP [LD] group had a negative score, which was significantly different from the positive scores of other groups (Figure 4). During this phase of the experiment, animals in group E performed better than those in the normal control and PTZ + DZP groups. The final analysis for long-term memory in the discrimination phase revealed a positive score for the MP [LD] and MP [HD] treated groups, in contrast to the PTZ control group, which displayed a negative score (Figure 5), indicating a decline in long-term recognition

memory due to structural hippocampal damage caused by epileptic seizures, as recorded in the histological analysis (Plate 2). These findings are consistent with previous research by Osim *et al.*, (2017), which demonstrated that long-term consumption of unripe plantain improved learning and memory in an experimental mouse model. Throughout this experiment, diazepam had a positive effect on the animals, indicating that it has no negative effect on learning and memory.

In conclusion, these results indicate that MP stem juice exhibited significant antiepileptic activity in reducing the detrimental effects of status epilepticus and the associated structural deficit. In addition, MP stem juice was associated with positive behavioural parameters that are indicative of an enhanced long-term recognition memory.

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REFERENCES

- Adamson, S.S. and Ganiyu, O. (2012). Aqueous Extracts from Unripe Plantain (*Musa paradisiaca*) Products Inhibit Key Enzymes Linked with Type 2 Diabetes and Hypertension in vitro. *Jordan J. Biol. Sci.* 5(4): 239-246.
- Agbo, B. E., Nta, A. I. and Ajaba, M. O. (2019). Bio-pesticidal Properties of Neem (*Azadirachta indica*). *Adv. Trends in Agric. Sci.*, Book Publisher International, 1:10-26. DOI:10.9734/bpi/atias/v1
- Agbo, B. E., Nta, A. I. and Ajaba, M. O. (2015). A Review on the Use of Neem (*Azadirachta indica*) as a Bio-pesticide, *J. Biopestic Environ.*, 2 (1):58-65
- Ajaba, M. O., Brooks, A. A. and Emanghe, U. E. (2016). Antimicrobial potency of neem (*Azadirachta indica*) leaf extracts on pathogenic bacteria isolated from wound infection. *Int. J. Sci. Eng. Res.* 7(1): 1501-1507.
- Akpabio, U. D., Udiong, D.S. and Akpakpan, A. E. (2012). The Physicochemical characteristics of plantain (*Musa paradisiaca*) and banana (*Musa sapientum*) pseudo stem wastes. *Adv. Nat. Appl. Sci.* 6 (2): 172.
- Akpuaka, M. U. and Ezem, S. N. (2011). Preliminary photochemical screening of some Nigerian dermatological plants. *J. Basic Phys. Res.* 2 (1): 5.
- Anani, S. E., Eru, E. M., Okon, D., Uruakpa, K. C., Ugbem, T. I., Igiri, A. O. (2020). The Effect of Averrhoa Carambola (Star Fruit) Aqueous Fruit Extract on the Hippocampal Astrocyte Expression following Diazepam-Induced Neurotoxicity in Wistar Rats. *Trop J Nat Prod Res.* 4 (12):1170-1173.
- Arellano, J. I., Muñoz, A., Ballesteros-Yáñez, I., Sola, R. G. and DeFelipe, J. (2004). Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus, *Brain.* 127 (1): 64.
- Bancroft, J. and Stevens, A. (1982). *Theory and Practice of Histological Techniques.* (2nd ed). New York, Churchill Livingstone. 131-135.
- Bazil, C. W., Morrell, M. J. and Pedley, T. A. (2005). *Epilepsy.* In: Rowland LP. (11th ed) *Merritt's Neurology.* Philadelphia; pp. 1008.
- Borges, M. H., Alves, D. L. F., Raslan, D. S., Pilo-Veloso, D., Rodrigues, V. M., Homs-Brandeburgo, M. I., De Lima, M. E. (2005). Neutralizing properties of *Musa paradisiaca* L. (Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of Crotalidae venoms. *Jour. Ethnopharmacol.*, 98 (1-2): 21-9.
- Broadbent, N. J., Gaskin, S., Squire, L. R. and Clark, R. E. (2010). Object recognition memory and the rodent hippocampus. *Learn Mem.*, 17:11
- Citraro, R., Navarra, M., Leo, A., Donato-Di-Paola, E., Santangelo, E., Lippiello, P., Aiello, R., Russo, E. and De Sarro, G. (2016). The Anticonvulsant Activity of a Flavonoid-Rich Extract from Orange Juice Involves both NMDA and GABA-Benzodiazepine Receptor Complexes. *Mol. (Basel, Switzerland)*, 21 (9); 1261.
- Denninger, J. K., Smith, B. M. and Kirby, E. D. (2018). Novel Object Recognition and Object Location Behavioral Testing in Mice on a Budget. *J Vis Exp.*, (141), e58593, 1-10.
- Díaz-Negrillo, A. (2013). Influence of sleep and sleep deprivation on ictal and interictalepileptiform activity. *Epilepsy Res Treat.*, 2013: 1-7, doi:10.1155/2013/492524.
- Epilepsy Foundation (2010). About epilepsy and seizures. Available at: www.epilepsyfoundation.org/about/statistics.cfm.
- Erkeç, O. E. and Arihan, O. (2015). Pentylentetrazole Kiindling Epilepsy Model. *Epilepsia*; 21(1):12.
- Eru, E. M., Anani, S. E., Paulinus, S. O., Mesembe, O. E., Uruakpa, K. C., Umoh, N. M., Oku, M. E., Akpaso, M. I., Igiri, A. O. (2022). Scopolamine-Induced Alzheimer's disease in Wistar Rats; Aqueous Talinum Triangulare Potency on the Hippocampal Nissl Bodies and Long-Term Learning and Memory. *Trop J Nat Prod Res.* 6 (1):117-122.
- Eru, E. M., Paulinus, S. O., Udo-Affah, G. U., Uruakpa, K. C., Oku, M. E., Anani, S. E., Umoh, N., Akpaso, M. I. and Igiri, A. O. (2021b). Hippocampal Astroglial Reduction in Scopolamine Hydrobromide-Induced Alzheimer's Cognitive Dysfunction Wistar Rats Following Administration of Aqueous Extract of Telfairia occidentalis (Hook F.) Seeds. *Niger. J. Physiol. Sci.* 36: 241 - 244 www.njps.physiologicalsociety.com
- Eru, E. M., Paulinus, S. O., Igiri, A. O. and Akpaso, M. I. (2020a). Enhanced Effect of Aqueous Extract of Telfaria occidentalis Seed on the Microstructure of the Hippocampus of Scopolamine Hydrobromide-Induced Cognitive Dysfunction Rats. *Asian. J. Res. Rep. Neurol*, 3 (1): 5-10.
- Eru, E. M., Paulinus, S. O., Udo-Affah, G. U., Uruakpa, K. C., Oku, M. E., Edet, U. I. and Igiri, A. O. (2020b). Neurobehavioral Enhancement in Scopolamine Hydrobromide-Induced Alzheimer Type Cognitive Dysfunction in Rats Following Administration of Aqueous Seed Extract of Telfaria occidentalis (Hook.f.) Cucurbitaceae. *Trop J Nat Prod Res.* 4(7):282-285.
- Eru, E. M., Udo-Affah, G. U., Paulinus, S. O., Uruakpa, K. C., Oku, M. E., Anani, S. E., Blessed, N. C., Nyiam, T., Umoh, N. M. and Igiri, A. O. (2021a). Restorative Potentials of Aqueous Telfaria occidentalis Seeds extract on the Hippocampal Nissl Granules and Short Term Memory in Scopolamine Hydrobromide-Induced

- Alzheimer's Type Cognitive Dysfunction Rats. *Trop. J. Nat. Prod. Res.* 5(1):182-187.
- Fischer, V. A., Bassey, I. F., and Fischer, C. E. (2019). Methanolic Leaf Extract of *Vernonia amygdalina* on the Testes of Streptozotocin Induced Diabetic Wistar Rats. *J Pharm Biol Sci.*, 14(3):84-88
- Fisher, R. S., Scharfman, H. E. and DeCurtis, M. (2014). How can we identify ictal and interictal abnormal activity? *Adv Exp Med Biol.*, 813:3-23.
- Fisher, R. S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P. and Engel, J. Jr. (2005). Epileptic seizures and epilepsy: Definitions proposed by the International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia.*, 46 (4): 470 - 472.
- Frantseva, M. V., Velazquez, J. L., Hwang, P. A. and Carlen, P. L. (2000). Free radical production correlates with cell death in an in vitro model of epilepsy. *Eur J Neurosci.*, 12 (4):1431- 1439.
- Goldberg, E. M. and Coulter, D. A. (2013). Mechanisms of epileptogenesis: A convergence on neural circuit dysfunction. *Nat. Rev. Neurosci.*, 14:349
- Guyton, A. C. and Hall, J. E. (2000). *Textbook of Medical Physiology.* (10th ed.) London: W. B. Saunders Company, pp. 198-216
- Hammond, N. (2016). Tonic-Clonic Seizures☆. Reference Module in Biomedical Sciences. doi:10.1016/b978-0-12-801238-3.995
- Harper, D. O. (2001). Meaning of epilepsy. *Brit Med Jour*, 2: 321.
- Holmes, G. and Ben-Ari, Y. (1998). Seizures in the developing brain: Perhaps not so benign after all. *Neuron.*, 21: 1234.
- Kim, J. H, Kang, J. W., Kim, M. S., Bak, Y., Park, Y.S., Jung, K. Y., Lim, Y. H. and Yoon, D. Y. (2012). The apoptotic effects of the flavonoid N101-2 in Human cervical cancers. *Toxicol In vitro*; 26 (1): 73
- Klioueva, I. A., Van Luijtelar, E. L., Chepurnova, N. E. and Chepurnov, S. A. (2001). PTZ-induced seizures in rats: Effects of age and strain. *Physiol. Behav.*, 72: 426.
- Kojovic, M., Cordivari, C. and Bhatia, K. (2011). Myoclonic disorders: a practical approach for diagnosis and treatment. *Ther Adv neurol Disord*, 4 (1), 47–62.
- Leone, M., Ciccacci, F., Orlando, S., Petrolati, S., Guidotti, G., Majid, N. A, Tolno, V. T., Sagno, J., Thole, D., Corsi, F. M., Bartolo, M. and Marazzi, M. C. (2021). Pandemics and Burden of Stroke and Epilepsy in Sub-Saharan Africa: Experience from a Longstanding Health Programme. *Int. J. Environ. Res. Public Health*, 18 (5), 2766.
- Lorke, D. G. (1983). A new approach to practical acute toxicity testing. *Arch Toxicol.*, 54:287.
- Loscher, W. (2002). Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res.*, 50:123.
- Lueptow, L. M. (2017). Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. *J V Exp.*, 126: 55718.
- Massey, C. A., Sowers, L. P., Dlouhy, B. J. and Richerson, G. B. (2014). Mechanism of sudden unexpected death in epilepsy: the pathway to prevention. *Nat Rev Neurol.*, 5: 282
- Mayer, S. A., Claassen, J., Lokin, J., Mendelsohn, F., Dennis, L. J., and Fitzsimmons, B. F. 2002. Refractory status epilepticus: frequency, risk factors, and impact on outcome. *Arch Neurol.*, 59(2), 205. doi:10.1001/archneur.59.2.205
- Meldrum, B. S. and Horton, R. W. (1973). Physiology of Status Epilepticus in Primates. *Arch Neurol.*, 28(1), 1–9. doi:10.1001/archneur.1973.00490190019
- Meldrum, B. S. (1973). Systemic Factors and Epileptic Brain Damage. *Arch Neurol.*, 29(2), 82. doi:10.1001/archneur.1973.0049026
- National Institute of Neurological Disorders and Stroke. (2015). "Epilepsy: Hope through Research". "Retrieved from" <https://www.ninds.nih.gov/>
- Niquet, J., Baldwin, R. A., Allen, S. G., Fujikawa, D. G. and Wasterlain, C. G. (2003). Hypoxic neuronal necrosis: Protein synthesis-independent activation of a cell death program. *Proc Natl Acad Sci U S A*, 100 (5), 2825–2830. doi:10.1073/pnas.0530113100
- Olajuyigbe, O. O. and Afolayan, A. J. (2011). Phytochemical Assesment and Antioxidant Activities of Alcoholic and Aqueous Extracts of *Acacia mearnsii* De Wild. *Int J Mol*, 7(8): 856-861
- Oliveira, A. M. M., Hawk, J. D., Abel, T. and Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learn Mem.*17: 160
- Olsen, R. W. (1981). GABA-benzodiazepine-barbiturate receptor interactions. *J Neurochem.*, 37(1): 1-13.
- Onyenekwe, P. C., Okereke, O. E. and Owolewa, S. O. (2013). Phytochemical screening and effect of *Musa paradisiaca* stem extrude on rat haematological parameters. *Curr Res J Biol Sci.*, 5 (1): 26-29.
- Osim, E., Peter, E., Ofem, O. and Okon, U. (2017). Chronic Consumption of Plantain (*Musa paradisiaca*) Diet Increases Learning and Memory in Mice. *Pharm Biol.*, 7(2): 64-68.
- Pearl, P. L. (2009). New treatment paradigms in neonatal metabolic epilepsies. *J Inherit Metab Dis* 32(2): 204-213.
- Pitkänen, A., Lukasiuk, K., Dudek, F. E. and Staley, K. J. (2015). Epileptogenesis. *Cold Spring Harb. Perspect Med* 5(10): 1-17.
- Ramanjaneyulu, R. and Ticku, M. K. (1984). Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. *Eur. J. Pharmacol.*, 98: 345.
- Reddy, A. J., Dubey, A. K., Handu, S. S., Sharma, P., Mediratta, P. K., Ahmed, Q. M. and Jain, S. (2018). Anticonvulsant and Antioxidant Effects of *Musa sapientum* Stem Extract on Acute and Chronic Experimental Models of Epilepsy. *Pharmacogn Res.*, 10(1): 49–54.
- Serra, L., Bozzali, M., Fadda, L., De Simone, M. S., Bruschini, M., Perri, R., Caltagirone, C. and Carlesimo, G. A. (2020). The role of hippocampus in the retrieval of autobiographical memories in patients with amnesic Mild Cognitive Impairment due to Alzheimer's disease. *J Neuropsychol.*, 14(1): 46-68.
- Shibasaki, H., and Hallett, M. (2005). Electrophysiological studies of myoclonus. *Muscle & Nerve.*, 31(2): 157–174. doi:10.1002/mus.20234

- Shields, W. D. (2006). Infantile spasms: little seizures, BIG consequences. *Epilepsy curr.*, 6(3):63-69.
- Shin, E. J., Jeong, J. H., Chung, Y. H., Kim, W. K., Ko, K. H., Bach, J. H., Hong, J. S., Yoneda, Y. and Kim, H. C. (2011). Role of oxidative stress in epileptic seizures. *Neurochem. Int*; 59(2):122-137.
- Singh, I. (2004). *Introduction to Human neuroanatomy*. (6th ed.). New Delhi: Jaypee Brothers Medical Publishers. pp. 25.
- Stachowicz, K., Branski, P., Klak, K., Van der Putten, H. and Cryan, J. F. et al. (2008). Selective activation of metabotropic G-protein-coupled glutamate 7 receptor elicits anxiolytic-like effects in mice by modulating GABAergic neurotransmission. *Behave. Pharmacol*; 19(5-6): 597-603.
- Sutula, T., Hagen, J. and Pitkanen, A. (2002). Do seizures damage the brain? (2002). *Curr Opin Neurol.*, 16(2): 189-195.
- Trinka, E., Cock, H., Hesdorffer, D., Rossetti, A. O., Scheffer, I. E., and Shinnar, S., Shorvon, S. And Lowenstein, D. H. (2015). A definition and classification of status epilepticus: report of the ILAE Task Force on Classification of Status Epilepticus. *Epilepsia*. 56(10):1515–1523. doi:10.1111/epi.13121
- Udoh, B. E., Erim, A. E., Paulinus, S. O., Eru, E. M, Ukpong, E. V., Efanga, I., Archibong, B. E., Egong, A. E., Udo, E. E., Egbe, N. O. (2020). Effects of *Cyperus esculentus* (Tiger Nut) Extract on the Irradiated Testes of Wistar Rats. *Trop J Nat Prod Res*. 4(11):966-969.
- University of Florida Herbarium, UFH, (2022). Preparation of Plant Specimens for Deposit as Herbarium vouchers. Retrieved July 12, 2022 from <https://www.floridamuseum.ufl.edu/herbarium/methods/vouchers/>
- World Health Organization. (2001). *Epilepsy: Aetiology, epidemiology and prognosis*. Fact sheet No.165. Retrieved 15-06-2009.
- World Health Organization. (2019)s. *Epilepsy: Signs and symptoms, rates of disease, causes, treatment, prevention, social and economic impacts, human rights and WHO response*. Fact sheet; pp. 51.

Full length Research Article

Modulatory effect of ethanol root extract of *Sarcocephalus latifolius* on Fertility of hypertensive Wistar rats induced by Nw-Nitro-L-Arginine Methyl Ester

Adejumobi O. A.¹, *Ajani O.S.², Faturoti J.T.¹, Adewoyin A.G.³, Ajani T.F.¹, Esan O.O.¹, Oyagbemi A.A.⁴, Omobowale T.O.¹, Adedapo A.A.⁵, Ohore O.G.⁶, Oyeyemi M.O.², Ashafa T.O.A.⁷, Yakubu M.A.⁸

¹Departments of ¹Veterinary Medicine, ²Theriogenology, ⁴Veterinary Physiology and Biochemistry and

⁶Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan.

³Department of Science Laboratory Technology, LAUTECH, Ogbomosho, Nigeria.

⁵Toxicology and Assessment Section, Michigan Department of Health and Human Services, USA.

⁷Department of Plant Science, University of the Free State, Qwaqwa, South Africa

⁸Department of Interdisciplinary studies, Texas State University, Houston, TX, U.S.A

Summary: This study was designed to investigate the modulatory effect of ethanol root extract of *Sarcocephalus latifolius* (SL) on the fertility of hypertensive Wistar rats induced by Nw-Nitro-L-Arginine Methyl Ester. Fifty adult male Wistar rats were randomly divided into 5 groups A-E. The rats in group A (Control) were administered with distilled water while Groups B-E received L-NAME at 40 mg/kg, Groups C, D, were co-administered SL at dosage of 100 and 200 mg/kg body weight, respectively, and group E was co-administered with Captopril 20 mg/kg once daily for 28 days. L-NAME caused a significant increase in blood pressure (mmHg) with Systolic Blood Pressure (SBP) (159.08±2.89), Diastolic Blood Pressure (DBP) (114.67±3.83) and Mean Arterial Pressure (MAP) (120.90±4.65) values when compared with their respective control of (115.00±2.81, 80.91±2.76 and 91.9±2.68) in Group B. The high blood pressure was however lowered in groups co-administered with SL and Captopril. Higher morphological alterations of sperm cells were observed in hypertensive rats and hypertensive rats medicated with captopril in this study, It was noticed that the right testicular weight and right testicular length in group C were affected significantly when compared to the left testicular parameter in groups A and B. Semen characteristics showed a decrease in sperm motility and liveability in hypertensive rats group compared to the control and extract treated groups. This decrease fell below acceptable 60 % minimum sperm motility recommended for breeding animals and percentage of the abnormal sperm cell in group B is higher than 20% maximum acceptable limit in normal breeding animals. Hypertension altered the reproductive indices in rats used for this study and could result in infertility but ethanol extract of *S. latifolius* ameliorated the reproductive organ damage in hypertensive rats.

Keywords: Hypertension, Infertility, rats, *Sarcocephalus latifolius*, Nw-Nitro-L-Arginine Methyl Ester

*Authors for correspondence: muyenko@yahoo.com, Tel: +234- 8033728003

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INTRODUCTION

Non-communicable diseases such as hypertension, a chronic condition of elevated blood pressure and risk factor for cardiovascular diseases have become a cause for global concern (Adeboye *et al.*, 2015) About a billion people worldwide are affected by hypertension otherwise referred to as the "silent killer" as it has no distinct sign and symptom at the initial stage (Marshall *et al.*, 2012) High blood pressure is defined as a systolic blood pressure at or above 140 mmHg and/or a diastolic blood pressure at or above 90 mmHg. Systolic blood pressure is the maximum pressure in the arteries when the heart contracts (Magder, 2018). The

economic burden of hypertension includes cardiovascular complications such as heart failure, hypertrophic cardiomyopathy, others include stroke and renal failure, (Zhou *et al.*, 2021).

Hypertension is associated with an increased incidence of sexual dysfunction (Lou *et al.*, 2023). The aetiology for male factor infertility is multifactorial. Most studies focus on sperm motility (SM), viability (SV), and DNA integrity since these parameters are important characteristics of sperm function (Sati and Huszar, 2015) Infertility in the male is the inability to produce fertile sperm cells or semen for fertilization of fertile ovum, which does not lead to

conception of the female bred (Magalhaes *et al.*, 2021). Male infertility may be induced by many factors which include diseases, exposure to environmental conditions, adverse drug effect or plants (Adewoyin *et al.*, 2017). One lifelong non-infectious disease with infertility as one of its sequels is hypertension. Several studies examining hypertensive men demonstrated a significant inverse relationship between blood pressure and total serum testosterone, which could be associated with impaired reproductive potential, free testosterone and sex hormone binding globulin (Narinx *et al.*, 2022, Svartberg *et al.*, 2004).

Studies have reported that N^w-Nitro-L-Arginine Methyl Ester (L-NAME)-induced hypertension has been associated with attenuated endothelium-dependent relaxations, cardiac and aortic tissue damage, renal vascular, and glomerular fibrosis (Francois *et al.*, 2004; Nyadjeu *et al.*, 2013).

Sarcocephalus latifolius belongs to the family of Rubiaceae, the common names include pin cushion tree or African peach. It is locally called “Egbesi” in Yoruba, “Ubuluino” in Igbo, “Marga” in Hausa and “Mbom-ibon” in Ibibio. *Sarcocephalus latifolius* is a small evergreen tree or straggling shrub with leaves rounded ovate, apex shortly acuminate, rounded or lunate base and stipulates ovates (Me *et al.*, 2016). Parts used include leaves, roots, stem and fruits. It has been found useful in folk medicine for treatment of malaria, hypertension, diarrhoea, tuberculosis, dysentery and as a laxative (Okiemy-Andissa *et al.*, 2004). *S. latifolia* has been reported to be effective at lowering blood pressure at a dose of 2.5 – 20 mg/kg in normotensive rats and 2.5 – 10 mg/kg in hypertensive rats. The fact that *S. latifolia* had blood pressure lowering effect seems to justify its use as antihypertensive agent by the traditional medicine practitioner. The extract also decreased the heart rate dose dependently. Magnitude of response produced by the extract of *S. latifolius* was higher in hypertensive rats than in normotensive rats (Nworgu, 2008).

The antioxidant activity of the ethanol and aqueous extracts of the *S. latifolius* fruit has been investigated by Osama *et al.* (2017) using the DPPH scavenging assay, which determines the reducing power of the extract compared with the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 540 nm and also for the visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Mensor *et al.*, 2001). The ethanol and aqueous extracts of *S. latifolius* exhibited a significant dose-dependent inhibition of DPPH activity. However, the aqueous extract seems to have shown better DPPH scavenging activity across the different concentrations except at the final dose where both extracts showed comparably significant inhibition when compared to the standard (ascorbic acid).

It was also reported that the leaf extract of *Sarcocephalus latifolius* possesses hypoglycaemic effect (Gidado *et al.*, 2008). Diabetes mellitus is the commonest non-communicable endocrine disease and is considered one of the leading causes of death all over the world. The presence of phytochemicals in high concentration accounts for the significant hypoglycemic effect of *Sarcocephalus latifolius*

(Ndukwe *et al.*, 2017). Despite the vast medicinal actions of *Sarcocephalus latifolius* documented in literature, little is known about its anti-hypertensive property in the male animals exposed to Nw-Nitro-L-Arginine Methyl Ester. Therefore, the current study was designed to examine the modulatory effect of ethanol root extract of *Sarcocephalus latifolius* on reproductive parameters of hypertensive Wistar rats induced by Nw-Nitro-L-Arginine Methyl Ester.

MATERIALS AND METHODS

Experimental Animals: Fifty male albino rats were obtained from a reputable breeder. Their weights range from 95 to 230 g and were kept in the animal house throughout the period of the experiment. The rats were kept in a well-ventilated cage at optimum temperature and 12 hours light/dark cycle. They were fed with commercial growers mash and water. The experiment was carried out following current guidelines established for the care of laboratory animals.

Experimental Design: Fifty adult male rats were randomly assigned into five different groups of ten animals each as follows: Group A serves as the control and was provided daily with feed and distilled water only. Group B was given 40 mg/kg of N^w-nitro-L-arginine-methyl ester (L-NAME) for 28 days via oral gavage while groups C was given 40 mg/kg of N^w-nitro-L-arginine-methyl ester and 100 mg/kg of *Sarcocephalus latifolius* root extract orally for 28 days, Group D: 40 mg/kg of N^w-nitro-L-arginine-methyl ester and 200 mg/kg of *Sarcocephalus latifolius* root extract orally for 28 days. Group E: was administered 40 mg/kg of N^w-nitro-L-arginine-methyl ester and 20 mg/kg of Captopril orally for 28 days.

Blood Pressure Measurement and Electrocardiography

The systolic, diastolic, and mean arterial blood pressures can be determined non-invasively in conscious animals by tail plethysinography using an automated blood pressure monitor, CODA SI, Kent Scientific Corporation, Connecticut, USA).

Sample Collections and Analysis

Blood collection and Haematology: Blood was taken from the retro-orbital plexus into heparinised vials; transferred to heparinised bottles and plain bottles for the collection of serum and plasma, respectively. The blood taken was analysed for blood parameters including, haemoglobin concentration, full blood count, total leukocyte and differential leukocyte counts, packed cell volume (PCV) as well as serum protein analysis and Albumin: Globulin ratio in serum. The animals were then humanised by cervical dislocation while the final weight, relative organ weight (organ weight/100g of body weight) of thymus, kidney, liver and spleen was determined for each animal.

Experimental Animals Sacrifice: The rats were sacrificed by cervical dislocation, the animals were quickly dissected, and the testes were immediately harvested, rinsed with distilled water, blotted with tissue paper, and weighed.

Gonadometrics: The testes, after being separated from the epididymis and weighed. The length and diameter of the

testes were measured with the use of Electronic Veiner Caliper as described by Ajani *et al.* (2021).

Semen Analysis: Semen sample was collected from the caudal epididymis through an incision made with a scalpel blade. The sperm motility was assayed by dropping semen sample and 2.9 % buffered Sodium Citrate on a warm glass slide and then covered with a glass slip to view under a light microscope as described by (Zemjanis 1970, Ajani *et al.*, 2021). The sperm liveability was evaluated using a drop of semen on a warm glass slide, then stained with one drop of warm Eosin-Nigrosin stain. A thin smear was thereafter made of the mixture of semen and was stained on another glass slide. The smear was air dried and viewed under the microscope and assayed according to Zemjanis (1970).

Histopathological Examination: The histopathology of the testes was done using the method described by Drury *et al.* (1976.). Testes were fixed in formalin and later embedded in paraffin wax. To perform histology of tissues 4 µm sections were prepared with the help of Microtome (Leica, RM 2145). These sections then were deparaffinized in xylene. Xylene was removed using 100 % ethanol and then hydrated through reducing concentrations of ethanol, the ethanol was later rinsed from the tissue section with running water and dried. They were stained with Haematoxylin and Eosin and viewed under light microscope.

Statistical analysis: The data obtained were presented as mean ± SD (standard deviation). All mean differences were considered significant at 5% level, therefore *P*-values less than 0.05 (*P*<0.05) were considered statistically significant at *p*<0.05 using version 2.3 statistical software for Microsoft Excel. Significant elemental concentration differences in plants samples were determined by analysis of variance (ANOVA).

RESULTS

The body weight changes in animals before and after treatments with extracts of *Sarcocephalus latifolius* are shown in Table 1.

Effect of *Sarcocephalus latifolius* root extract on Haematological parameters: There was an increase in the PCV (%) of rats in all the treatment groups (35.2±4.86,

36.2±6.14 and 33.6±3.91) when compared to the control group (32±1.87). The mean total red blood cell counts, and haemoglobin concentration (Hb) also increased, while MCV, MCH and MCHC were significantly unchanged (Table 2)

Effect of *Sarcocephalus latifolius* root extract on Blood pressure profile of rats: There were significant (*P*<0.05) increases in the systolic, diastolic and mean arterial blood pressure of the rats given L-NAME alone (Hypertensive group) when compared to the control, extract treated and captopril treated groups. Also, a significant (*P*<0.05) decrease was observed in the blood pressure of rats treated with the *S. latifolius* extracts at 100 mg/kg and 200 mg/kg body weight. Likewise, there was a significant (*P*<0.05) decrease in the systolic and diastolic blood of the hypertensive rats treated with captopril when compared with those of hypertensive group (B) and hypertensive extract treated groups (C and D) at the respective doses of 100 mg/kg and 200 mg/kg (Table 3)

Table 1:

Percentage increase in body weight before dozing till time of sacrifice

Treatment	Initial weight (g)	Final weight (g)	% Increase /Decrease
Control (-ve)	188.29	258.5	37.28
L-NAME	166.61	170.61	2.4
L-NAME + (100 mg/Kg extract)	138.41	173.89	25.64
L-NAME + (200 mg/Kg extract)	150.73	158.21	4.96
L-NAME + Captopril	178.48	165.88	-7.0 ^a

Effect of *Sarcocephalus latifolius* root extract on sperm motility and liveability: A significant (*P*<0.05) reduction was observed in the motility in motility and liveability of the spermatozoa of hypertensive rats compared to the control. On the other hand, a significant (*P*<0.05) increase was observed in the sperm motility and liveability of hypertensive rats treated with *S. latifolius* extract and captopril when compared with those of the hypertensive group (B) (Table 4).

Table 2:

Hematological parameters of rats treated with or without *Sarcocephalus latifolius* root extract and L-NAME

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
PCV (%)	32±1.87	32.4±3.64	35.2±4.86	36.2±6.14	33.6±3.91
Hb (dL)	10.4±0.30	10.76±1.24	11.66±1.64	12.02±2.07	11.14±1.32
RBC (X10 ⁶ /µL)	10.07±1.64 ^c	12.31±2.65	15.16±1.31	13.3±1.09	13.38±0.84
WBC (X10 ⁶ /µL)	10.84±1.86	10.92±1.67	9.96±1.62	9.8±2.48	11.84±1.69
Platelet (10 ³ / µL)	10±0.0	9.6±0.89	10.4±0.89	10.4±1.67	10.4±0.89
MCV (fL)	31.6±4.27	24.4±2.88	23.6±5.98	26.8±3.27	24.8±2.58
MCH (pg)	10±1.41	7.8±0.83	7.6±1.94	8.4±1.14	8±1
MCHC (%)	33±0	33±0	33±0	33±0	33±0
LYMPH (%)	41.4±1.81	39.6±2.07	37.6±1.51	37.6±2.40	39±3.08
NEUT (%)	58.4±2.19	60.2±1.78	61.6±1.51	62.2±2.58	60.6±3.20
MONO (%)	0.2±0.44	0.2±0.44	0.8±1.09	0.2±0.44	0.4±0.54

Significance was at *P*<0.05, Means with superscript a, b showed significant differences when compared to the control and hypertensive groups, respectively

Modulatory Effects of *Sarcocelalus latifolius* on Fertility of Hypertensive Wistar Rats

Table 3:Blood Pressure (mmHg) profile of rats treated with or without *Sarcocephalus latifolius* root extract and L-NAME

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
Systolic	115±2.81 ^{bcd}	159.08±2.89 ^{cde}	127.67±1.70 ^{abe}	127±0.98 ^{abe}	118.5±2.44 ^{bcd}
Diastolic	80.91±2.76 ^b	114.67±3.83 ^{acde}	80.17±7.62 ^b	88.58±4.69 ^b	83.08±2.17 ^b
MAP	91.9±2.68 ^b	120.9±4.65 ^{cde}	96.33±5.35 ^b	100.75±3.04 ^b	95.33±2.22 ^b

Significance was at $P < 0.05$. Means with superscript a, b showed significant differences when compared to the control and hypertensive groups, respectively.

Table 4

Sperm Motility and Liveability of Experimental Groups

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
Sperm motility	66±27.93 ^a	46±26.07 ^a	82.8±25.16 ^a	85.2±20.08 ^a	90.8±12.13 ^a
Sperm liveability	88.8±13.08 ^a	64±19.49 ^a	81.2±20.80 ^a	85.2±20.08 ^a	91.6±3.58 ^a

Significance was at $P < 0.05$. Means with the same superscript are not statistically different at $P < 0.05$

Table 5:

Morphological abnormalities of Sperm cells of experimental groups rats

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
HWT	4 (0.8%) ^a	6 (1.2%) ^a	5 (1%) ^a	15 (3%) ^a	33 (6.6%) ^a
TWH	12 (2.4%) ^a	31 (6.2%) ^a	30 (6%) ^a	18 (3.6%) ^a	42 (8.4%) ^a
CT	42 (8.4%) ^a	79 (15.2%) ^a	38 (7.6%) ^a	16 (3.2%) ^a	72 (14.4%) ^a
LT & MP	3 (0.6%) ^a	12 (2.4%) ^a	1 (0.2%) ^a	5 (1%) ^a	51 (10.2%) ^a
BENT MP	0 (0%) ^a	4 (0.8%) ^a	0 (0%) ^{ab}	1 (0.2%) ^a	0 (0%) ^b
BENT TAIL	0 (0%) ^a	2 (0.4%) ^a	1 (0.2%) ^a	1 (0.2%) ^b	4 (0.8%) ^a
TOTAL	61 (12.2%) ^a	134 (26.8%) ^a	75 (15%) ^a	56 (11.2%) ^a	202 (40.4%) ^a

Significance was at $P < 0.05$. Means with the same superscript are not statistically different at $P < 0.05$.

HWT = Head without Tail, TWH = Tail without Head, CT = Curved Tail, LT & MP = looped Tail and Mid-piec

Table 6:Gonadometric indices of rats treated with or without *Sarcocephalus latifolius* root extract and L-NAME

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
Left testis weight	0.9±0.50 ^a	1.16±0.35 ^a	1.04±0.15 ^a	1.04±0.17 ^b	1.18±0.08 ^a
Right testis weight	1.14±0.05 ^a	1.2±0.25 ^a	1.14±0.08 ^b	1.06±0.15 ^a	1.16±0.05 ^a
Left testis length	11.55±9.21 ^a	20.08±2.13 ^a	19.15±0.97 ^a	18.78±1.06 ^a	19.84±0.18 ^b
Right testis length	15.11±6.42 ^a	19.27±1.88 ^a	18.18±0.75 ^b	18.88±1.18 ^{ab}	18.88±1.25 ^a
Left testis diameter	7.23±4.28 ^a	9.85±0.57 ^a	9.11±1.27 ^a	9.56±0.36 ^b	9.47±0.54 ^{ab}
Right testis diameter	9.72±1.06 ^a	9.73±0.68 ^{ab}	9.07±0.60 ^a	8.96±0.57 ^a	9.44±0.41 ^a

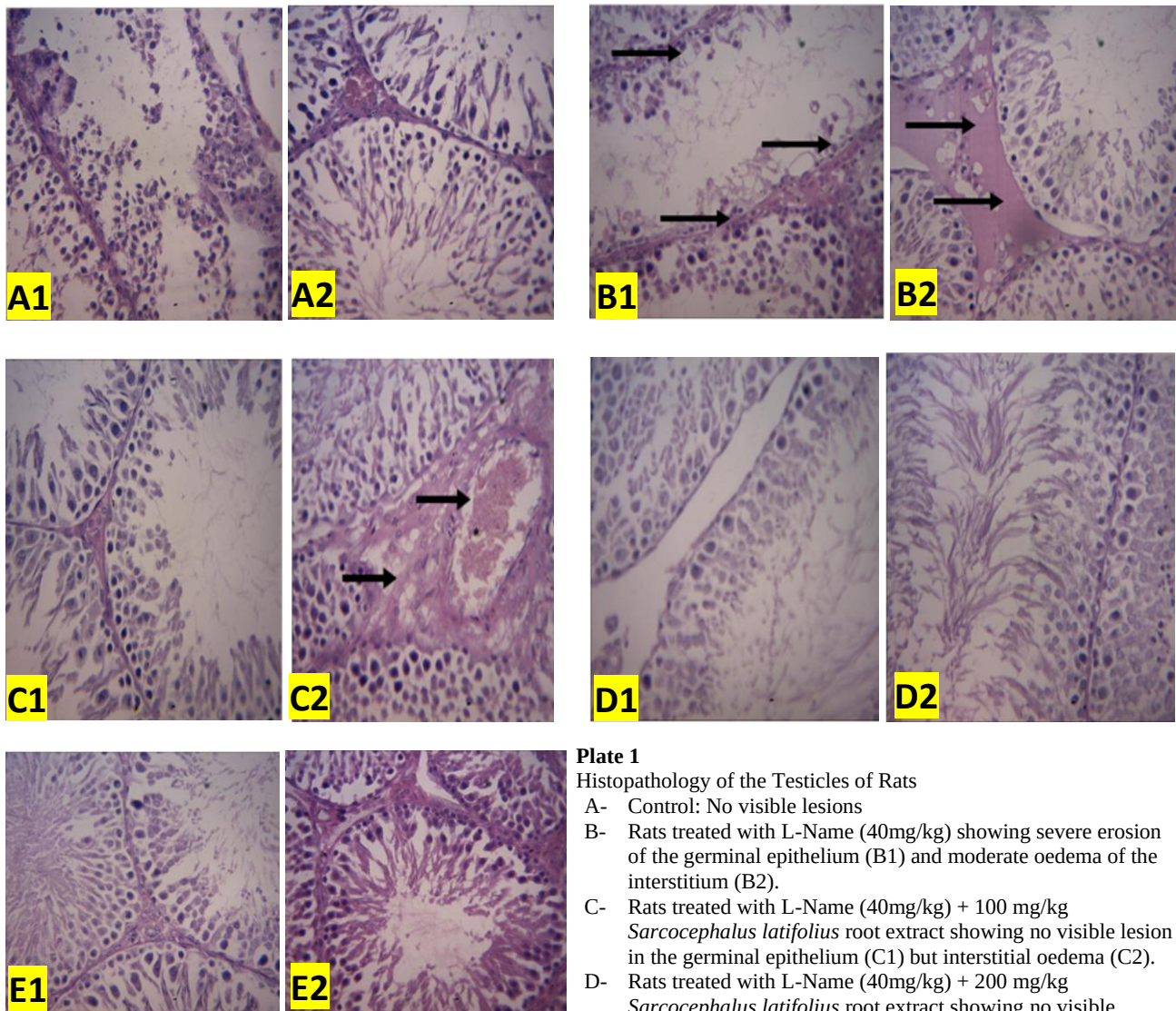
Significance was at $P < 0.05$. Means with the same superscript are not statistically different at $P < 0.05$

Effect of *Sarcocephalus latifolius* root extract on Sperm morphology: The percentage of sperm morphological abnormalities were significantly higher ($P < 0.05$) in hypertensive rats when compared with the control, normotensive rats and extract treated rats. The percentage sperm morphological abnormalities of the rats treated with 200 mg/kg extract was observe to have the least abnormalities when compared with the control, captopril treated and the other extract treated groups while that of captopril medicated group had higher abnormality percentage when compare to the hypertensive and extract treated groups. (Table 5).

Effect of *Sarcocephalus latifolius* root extract on Gonadometric indices of rats with or without L-NAME

The gonadometric indices in hypertensive rats were slightly higher in hypertensive rats than the control group. However, there were no significant differences ($P > 0.05$) in the indices of extract and captopril treated groups when compared to the hypertensive group (Table 6).

Effect of *Sarcocephalus latifolius* root extract on the histopathology of the Testicles of Rats: The results of the histopathological investigations of the testis of the animals are shown in Plate 1.

**Plate 1**

Histopathology of the Testicles of Rats

- A- Control: No visible lesions
- B- Rats treated with L-Name (40mg/kg) showing severe erosion of the germinal epithelium (B1) and moderate oedema of the interstitium (B2).
- C- Rats treated with L-Name (40mg/kg) + 100 mg/kg *Sarcocephalus latifolius* root extract showing no visible lesion in the germinal epithelium (C1) but interstitial oedema (C2).
- D- Rats treated with L-Name (40mg/kg) + 200 mg/kg *Sarcocephalus latifolius* root extract showing no visible lesions.
- E- Rats treated with L-Name (40mg/kg) + 20 mg/kg Captopril showing no visible lesions.

DISCUSSION

This study was carried out to investigate the modulatory effects of ethanol root extract of *Sarcocephalus latifolius* on the fertility potential of hypertensive male Wistar rat induced by L-NAME. Our findings in this study showed that the L-NAME treated group became hypertensive while the groups co-treated with *S. latifolius* and captopril had their blood pressure reduced to normal values comparable to that of the control group. This finding is supported by the report of Nworgu *et al.* (2008) that *S. latifolius* has blood pressure lowering effect in rats.

Findings from this study showed that packed cell volume and red cell indices of all hypertensive rats treated with *S. latifolius* increased significantly ($p < 0.05$). This shows that *S. latifolius* has haemopoietic potential. This finding agreed with Saba *et al.* (2009) study on the fruit extract, where the treated groups demonstrated to possess haematopoietic effect of the extract. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly different, which indicate that the red cells were

matured cells. This homogeneity in size may be due to the period of the study which was long enough for hematopoiesis to occur and maturation of the cells.

It was noticed that the left testicular weight, length and diameters in groups B, C, D, and E were significantly different (< 0.05) when compared to those of their respective control group. The right testicular diameter also increased on groups B, C, D, and E when compared to the control. However, there were no significant differences in the right testicular weights and diameters across all the treatment groups and control. This indicates that left testes were more affected by hypertension and treatments with *S. latifolius* extract and captopril. This agrees with a study that reported that hypertension is associated with an increased incidence of sexual dysfunction (Lou *et al.*, 2023). Some researchers also found out that lower semen volume, sperm mobility, sperm count and total motile sperm count were associated with hypertension (Guo *et al.*, 2017). Anti-hypertensives were also shown to have adverse effects on fertility (Guo *et al.*, 2015). Angiotensin Converting Enzyme Inhibitors were reported to cause relatively decreased semen volume and decreased sperm motility (Guo *et al.*, 2015).

Semen characteristics showed a decrease in sperm motility in group B. This decrease falls below acceptable 60 % minimum for sperm motility for a breeding animal (Palmer *et al.*, 2013). Also the liveability value showed a decrease in group B when compared to other group. Since motility and liveability determine fertility, findings from this study therefore showed that hypertension may cause infertility problem in male rats if left untreated (Eisenberg *et al.*, 2016). The extract of *S. latifolius* however improved the sperm motility and liveability in hypertensive rats. It shows that *S. latifolius* has a great reproductive modulatory potential. This agrees with the report of Balogun *et al.* (2016) that *S. latifolius* has diseases prevention and curative effects through antioxidant, anti-inflammatory, antihypertensive, anti-secretory and anti-ulcerative mechanisms.

The percentage of the abnormal sperm cell in group B and E were higher than 20% maximum acceptable in normal sperm breeding animal. Therefore, group B and E having 26.8% and 40.4 % respectively are too high. This findings is similar to Saba *et al.* (2009) who observed sperm abnormality of rats in treatment group were significantly ($P < 0.05$) higher in relative to the control rats. This observation is supported by histological findings of the testes of the hypertensive rats which exhibit erosion of the germinal epithelium and interstitial oedema. The histology of the group treated with captopril also revealed severe congestion and interstitial eodema in the section. This findings is supported by the fact that hypertension cause organ damage and some antihypertensive may fail to reverse the damage caused by hypertension or they have adverse effects on the reproductive organs (Eisenberg *et al.*, 2016; Guo *et al.*, 2015). The extract treated groups however did not show any abnormality on the histology of the testes. This showed the ameliorative effect of the extract on the testicular damage caused by hypertension.

In conclusion, this study showed that treatment with *S. latifolius* extract reduced the blood pressure and ameliorated the testicular damage and sperm abnormality caused by hypertension.

REFERENCES

- Adeboye, P. T., Bettiga, M., Aldaeus, F., Larsson, P. T., & Olsson, L. (2015). Catabolism of coniferyl aldehyde, ferulic acid and p-coumaric acid by *Saccharomyces cerevisiae* yields less toxic products. *Microbial cell factories*, 14(1): 1-14.
- Adewoyin, M., Ibrahim, M., Roszaman, R., Md Isa, M. L., Mat Alewi, N. A., Abdul Rafa, A. A., & Anuar, M. N. N. (2017). Male infertility: the effect of natural antioxidants and phytochemicals on seminal oxidative stress. *Diseases*, 5(1): 9.
- Aghi, M., Hochberg, F., & Breakefield, X. O. (2000). Prodrug activation enzymes in cancer gene therapy. *The journal of gene medicine*, 2(3): 148-164.
- Ajani, O. S., Oyeyemi, M. O., & Olukowi, M. O. (2020). Fertility effects of *Anacardium occidentale* (Cashew leaves) in the West African Dwarf bucks. *Nigerian Veterinary Journal*, 41(4): 331-342.
- Balogun, O. O., O'Sullivan, E. J., McFadden, A., Ota, E., Gavine, A., Garner, C. D. & MacGillivray, S. (2016). Interventions for promoting the initiation of breastfeeding. *Cochrane Database of Systematic Reviews*, 11: 1-10
- Bobrie, G., Chatellier, G., Genes, N., Clerson, P., Vaur, L., Vaisse, B., et al. (2004) Cardiovascular prognosis of "masked hypertension" detected by blood pressure self-measurement in elderly treated hypertensive patients. *JAMA*. 291: 1342-9.
- Burchardt, M., Buchardt, T., Baer, L., Kiss, A.J., Pawar, R.V., Shabsigh, A., De La Taille, A., Hayek, O.R. And Shabsigh, R. (2000). Hypertension is associated with severe erectile dysfunction. *The Journal of urology*, 164 (4): 1188-1191.
- Drury R.A., Wallington E.A., Cancerson R. (1976) *Calton's histopathological techniques* (4th edition) UK, Oxford University Press.
- Eisenberg, M.L, Li S., Behr, B, Pera, R.R., Cullen M.R. (2015). Relationship between semen production and medical comorbidity. *Fertil Steril* 103: 66–71.
- Eisenberg, M.L., Li, S., Cullen, M.R., and Baker, L.C. (2016). Increased risk of incident chronic medical conditions in infertile men: analysis of United States claims data. *Fertil Steril*. 105: 629–636.
- Francois, H., Placier, S., Flamant, M. et al., (2004). "Prevention of renal vascular and glomerular fibrosis by epidermal growth factor receptor inhibition," *The FASEB Journal*, 18(7): 926– 928.
- Gidado, A., Ameh, D. A., Atawodi, S. E., & Ibrahim, S. (2008). Hypoglycaemic activity of *Nauclea latifolia* Sm. (Rubiaceae) in experimental animals. *African Journal of Traditional, Complementary and Alternative Medicines*, 5(2): 201-208.
- Guo, D., Li, S., Behr, B. and Eisenberg, M. (2015). The impact of hypertension and antihypertensives on semen quality. *J Urol*. 193: 1117.
- Guo, D., Li, S., Behr, B., and Eisenberg, M. L. (2017). Hypertension and Male Fertility. *The world journal of men's health*, 35(2): 59–64.
- Kung, A. W. C., Pang, R. W. C., & Janus, E. D. (1995). Elevated serum lipoprotein (a) in subclinical hypothyroidism. *Clinical endocrinology*, 43(4): 445-449.
- Lou, I. X., Chen, J., Ali, K., & Chen, Q. (2023). Relationship Between Hypertension, Antihypertensive Drugs and Sexual Dysfunction in Men and Women: A Literature Review. *Vascular Health and Risk Management*, 691-705.
- Magalhães, J. A., Ribeiro, L. S., Rego, J. P. A., & de Andrade, C. R. (2021). Current markers for infertility in men. *JBRA Assisted Reproduction*, 25(4): 592.
- Magder, S. (2018). The meaning of blood pressure. *Critical Care*, 22: 1-10
- Mahdi, M. A., Shwish, D. L., & Almhmd, N. A. (2018). Evaluation of the impact of the level of (Testosterone, Luteinizing Hormone, Follicle-Stimulating Hormone and Prolactin) on some semen parameters in infertile males. *Tikrit Journal of Pure Science*, 23(7): 27-31.
- Marshall, N. A., Park, S. E., Adger, W. N., Brown, K., & Howden, S. M. (2012). Transformational capacity and the influence of place and identity. *Environmental Research Letters*, 7(3): 034022.
- ME, B., Besong, E. E., Obu, D. C., Obu, M. S. U., & Djobissie, S. F. A. (2016). *Nauclea latifolia*: a medicinal, economic and pharmacological review. *International Journal of Plant Research*, 6(2), 19.
- Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C.D., Coube, C.S. and Leitão, S.G., (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, 15(2): 127-130.
- Muciaccia, B., Pensini, S., Culasso, F., Padula, F., Paoli, D., and Gandini, L. et al. (2012). Higher cluster in

- immunolabeling and sperm DNA damage levels in hypertensive men compared with controls. *Hum Reprod.* 27:2267–2276.
- Narinx, N., David, K., Walravens, J., Vermeersch, P., Claessens, F., Fiers, T., AntonioL & Vanderschueren, D. (2022). Role of sex hormone-binding globulin in the free hormone hypothesis and the relevance of free testosterone in androgen physiology. *Cellular and Molecular Life Sciences*, 79(11): 543.
- Ndukwe, M., Adoga, G., & Luka, C. (2017). Effect of Ethanolic Leaf Extract of *Sarcocephalus latifolius* on Some Biochemical Parameters in Alloxan Induced Diabetic Rats. *Journal of Advances in Medical and Pharmaceutical Sciences*, 14(4): 1-7.
- Nwankwo, T., Yoon, S.S., Burt, V. and Gu, Q. (2013). Hypertension among adults in the United States: National Health and Nutrition Examination Survey, 2011-2012. *NCHS Data Brief.* (133): 1–8.
- Nworgu, D., Onwukaeme, N., Afolayan, A.J., Ameachina1, F.C. and Ayinde, B.A. (2008). Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *African Journal of Pharmacy and Pharmacology*. Vol. 2(2): 037-041
- Nyadjeu, P., Nguelefack-Mbuyo, E. P., Atsamo, A. D., Nguelefack, T. B., Dongmo, A. B. and Kamanyi, A. (2013). “Acute and chronic antihypertensive effects of *Cinnamomum zeylanicum* stem bark methanol extract in L-NAME-induced hypertensive rats,” *BMC Complementary and Alternative Medicine*, 13: 27.
- Okiemy-Andissa, N., Miguel, M.L., Etou, A.W., Ouamba, J.M., Gbeassor, M. and Abena, A.A. (2004). Analgesic effect of aqueous and hydroalcoholic extracts of three Congolese medicinal plants: *Hyptissuavolens*, *Nauclea latifolia* and *Ocimum gratissimum*. *Pakistan Journal Biological Science*. 7: 1613-1615.
- Osama, A., Awadelkarim, S. and Ali, A. (2017) Antioxidant activity, acetyl cholinesterase inhibitory potential and phytochemical analysis of *Sarcocephalus latifolius* sm Bark used in traditional medicine in Sudan. *BMC Complementary Alternative Medicine* 17: 270
- Palmer, C.W., Persson, Y. and Söderquist, L., (2013). Classification of the potential breeding ability of range beef bulls based on semen quality parameters in samples collected by transrectal massage—A comparison of the Swedish and Canadian systems. *Animal reproduction science*, 140(3-4): 124-130.
- Ritchey, M., Tsipas, S., Loustalot, F., & Wozniak, G. (2016). Use of pharmacy sales data to assess changes in prescription- and payment-related factors that promote adherence to medications commonly used to treat hypertension, 2009 and 2014. *PLoS One*, 11(7), e0159366.
- Saba, A.B., Oridupa, O.A., Oyeyemi, M.O., Osanyingbe, O.D. (2009). Spermatozoa morphology and characteristics of male Wistar rats administered with ethanolic extract of *Lagenaria breviflora* Roberts. *Afr J Biotech.* 8(7): 1170-1175.
- Sati, L., & Huszar, G. (2015). Sperm motility and viability: Overview of the cellular and physiological aspects that support these functions. *Eur Med J Repro Health*, 1(1); 74-80.
- Svartberg, J., Von Mühlen, D., Schirmer, H., Barrett-Connor, E., Sundfjord, J. and Jorde, R. (2004). Association of endogenous testosterone with blood pressure and left ventricular mass in men. The Tromsø Study. *Eur J Endocrinol.* 150: 65–71.
- Zemjanis, R. (1970). Collection and evaluation of semen. In: *Diagnostic and Therapeutic Techniques in Animal Reproduction*. William and Wilkins company, Baltimore, USA, 242.
- Zhou, B., Perel, P., Mensah, G. A., & Ezzati, M. (2021). Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. *Nature Reviews Cardiology*, 18(11);, 785-802.

Short communication

Hematopoietic Alteration in Carbon tetrachloride-Induced Liver Injury and Amelioration by *Vitex agnus-castus* Extract

***Ajani R.S. and Akpovwovwo N.A.**

Division of Gastrointestinal and Morphological Anatomy, Department of Anatomy, College of Medicine,
University of Ibadan, Nigeria.

Summary: Although the liver is not a primary hematopoietic site after the period of embryogenesis, however liver diseases in human can have adverse effects on hematopoiesis. This study evaluated the ability of *Vitex agnus-castus* extract to ameliorate hematopoietic alteration occasioned by carbon tetrachloride (CCl₄) induced acute liver injury. Varying doses of *V. agnus-castus* plant extract were administered to groups of rats with CCl₄ Induced acute liver injury. The low dose group (LEL) had 200mg/Kg body weight; the medium dose (MEL) had 400 mg/kg while the high dose group (HEL) had 600 mg/Kg of the extract once daily for 21 days. Each group had composite control without liver injury ie LE, ME and HE. The packed cell volume (PCV), hemoglobin concentration (Hb%), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were similar across the groups. Groups LEL and ME had significantly elevated white blood cell count (WBC). Amongst the composite groups ie LE: LEL; ME:MEL; & HE:HEL, there was significant difference within the composite group. The WBC differential was lymphocyte predominant without significant difference. The two low extract groups (LE & LEL) had significant thrombocytopenia ie low platelet count. The ethanolic extract of *V. agnus-castus* only affects the white cell and platelet components of the blood and not the erythrocyte parameters.

Keywords: Carbon tetrachloride- induced liver injury, *Vitex agnus castus* extract, red blood cell, white blood cell.

*Authors for correspondence: rs.ajani@mail1.ui.edu.ng rsaajani@yahoo.co.uk, Tel: +234-8023007362

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INTRODUCTION

The human circulatory system consists of the blood, lymph, body fluids and electrolytes, blood vessels, lymphatics and the heart. The two main components of the blood are cells and plasma which is fluidly. The cellular component of the blood consists of leukocytes (white blood cells), erythrocyte (red blood cell) and platelets. All these cell series have specific and diverse functions with structural or physiologic alterations resulting in a myriad of pathologies with variable dimensions. Hematopoiesis is the process by which these cells are formed.

During embryogenesis, hematopoiesis starts in the yolk sac mesoderm and subsequently involves the liver and spleen. Postnatally, hematopoiesis involves the stem cells precursors located in the bone marrow of the long bones, however, lymphocyte production also involves the lymphoid organs such as spleen, lymph nodes and spleen. Hematopoiesis can be altered by nutritional derangement, bone marrow diseases, chemical/drug toxicity, exposure to ionizing radiation, abnormal renal function and liver diseases. Deranged hematopoiesis may result in either low or elevated values of all or specific blood cells depending on the underlying pathology. Low cell counts may require transfusion of whole blood or its component, iron (ferrous) supplement or even bone marrow transplant. While

exchange blood transfusion or chemotherapy may be needed to correct elevated levels of blood cells counts.

Many chemical substances such as paracetamol, thioacetamide and carbon tetrachloride (CCl₄) have been used to induce acute liver injury in experimental animals (Butterworth *et al.*, 2009), the most widely used being CCl₄ (Cong *et al.*, 2017; Zhang *et al.*, 2017). The hepatic cytochrome P450 enzyme system metabolizes CCl₄ to produce highly active trichloromethyl radicals and reactive oxygen species (Wei *et al.*, 2023). All these molecules will lead to lipid peroxidation, oxidative stress and along with up regulation of proinflammatory cytokines, acute liver injury becomes established.

Vitex agnus-castus, a deciduous shrub that grows naturally in several countries including Nigeria has several phytochemicals such as flavonoids and diterpenoids that had been documented to have antioxidant activity (Hajdú Z *et al.*, 2007) and oestrogenic activity (Jarry H *et al.*, 2003) while its essential oils said to be bactericidal (Senatore F *et al.*, 2003). Other bioactivities attributable to the plant in literature include anti-inflammatory, antihistamine, analgesia and cytotoxicity against certain cell lines (Kamal N, *et al.*, 2022).

There is however no available information documenting its role in hematopoietic alteration occasioned

by carbon tetrachloride liver toxicity. This study was thus premised on this observation.

MATERIALS AND METHODS

Plant collection and authentication: *Vitex agnus castus* plant was sourced from a herbal garden located in Aladja, Delta State, South-South Region of Nigeria. Botanical identification and specie confirmation were done at the Herbarium Unit of the Department of Botany, University of Ibadan, Nigeria. For reference purpose, a sample of the plant was banked with the Herbarium with voucher number UIH-22953.

Extract preparation: The flowering stems of *V. agnus castus* plant were initially washed under running water and then allowed to dry under ambient temperature till moisture content was zero. The dried sample was subsequently milled into fine powder 1.2 kg of which was used for the ethanolic extraction with a 9.6 % yield. A portion of the powdery sample was used for phytochemical analyses.

Animals: Forty adult male Wistar rats weighing 190 to 290 g were sourced from the Central Animal house of the College of Medicine, University of Ibadan. They were acclimatized for three weeks in a well-ventilated and illuminated environment with optimal ambient temperature ($26\pm 2^{\circ}\text{C}$, 12 hours light / dark cycle) that was conducive for the study. The animals were fed liberally with locally sourced but standard pelletized rat feed and had unrestricted water intake.

Design of the Experiment: The confounding factors of the study were induction of liver injury and dosage of extract administered consequent upon which eight groups with five animals each were created as follows: (1) Normal control (NC)- liver injury not induced; (2) Liver injury (LI)- extract not administered; (3a) Low extract without liver injury (LE); (3b) Low extract with liver injury (LEL) ; (4a) Medium extract without liver injury (ME); (4b) Medium extract with liver injury (MEL); (5a) High extract without liver injury (HE) (5b) High extract with liver injury (HEL)

Induction of Liver Injury: Based on empirical evidence from available literature, liver injury was induced by single intraperitoneal administration of carbon tetrachloride at a dose of 1.6mg /kg (Maiti K *et al.*, 2005; Ohta Y *et al.*, 2000). The induction of acute liver injury was done for animals in groups LI, LEL, MEL and HEL.

Conduct of the Experiments: The ethanolic extract of the plant was administered once daily via oral steel canula to the following groups- low dose extract (LE & LEL), medium dose extract (ME & MEL) and High dose extract (HE & HEL) at respective dosage of 200 mg/kg, 400 mg/kg and 600 mg/kg for 21 days. The normal control (NC) and the liver injury (LI) groups had only rat chow and water for the same period. On day 22 of the study, venous blood was collected through intraocular puncture from the animals and stored in the appropriate specimen bottle for subsequent hematological analysis.

Ethical Conduct: In the conduct of this study, the animals were handled in accordance to the guidelines as prescribed by the ethical conduct of animal research of the University of Ibadan. Also, the principles of laboratory animal care as contained in the 8th edition (2011) of the Guide for the Care and Use of Laboratory Animals by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals were observed (16).

Data Analysis and Processing: The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 24 and expressed as percentages, means plus standard deviation of means (SD). The student t- test was used for inter group comparison and level of significance was set at $p < 0.05$.

RESULTS

The results of the haematological changes in control and extract treated rats are shown in Table 1. The packed cell volume (PCV) was least in the high extract dose (HE) group ($49.2\pm 3.19\%$) and highest in the liver injury (LI) group ($53.2\pm 2.23\%$). The differences in the PCV between the groups were insignificant.

Table 1:
Haematological Parameters

TESTS	GROUPS							
	CN	LI	LE	LEL	ME	MEL	HE	HEL
PCV(%)	52.6 $\pm$ 1.85	53.2 $\pm$ 2.23	50.0 $\pm$ 0.1	51.25 $\pm$ 2.59	51.75 $\pm$ 2.05	51.4 $\pm$ 4.27	49.2 $\pm$ 3.19	50.25 $\pm$ 2.28
Hb (g/dl)	17.1 $\pm$ 0.31	17.3 $\pm$ 0.42	16.6 $\pm$ 0.8	17.10 $\pm$ 0.84	16.94 $\pm$ 0.61	17.21 $\pm$ 0.9	16.3 $\pm$ 1.4	16.81 $\pm$ 0.6
RBC ($10^6/\mu\text{L}$)	8.61 $\pm$ 0.11	8.72 $\pm$ 0.12	8.23 $\pm$ 0.2	8.51 $\pm$ 0.31	8.71 $\pm$ 0.14	8.52 $\pm$ 0.31	8.32 $\pm$ 0.62	8.51 $\pm$ 0.31
MCV(μ^3)	60.82 $\pm$ 1.72	60.78 $\pm$ 2.17	59.44 $\pm$ 1.25	60.44 $\pm$ 2.15	59.35 $\pm$ 1.95	60.69 $\pm$ 3.09	59.58 $\pm$ 1.11	59.44 $\pm$ 0.91
MCHC (g/dl)	32.51 $\pm$ 0.67	33.04 $\pm$ 0.33	31.53 $\pm$ 0.32	32.57 $\pm$ 0.34	31.83 $\pm$ 1.76	33.10 $\pm$ 0.55	33.51 $\pm$ 0.6	32.53 $\pm$ 0.73
MCH (μg)	19.97 $\pm$ 0.14	19.87 $\pm$ 0.3	18.76 $\pm$ 0.48	19.96 $\pm$ 0.52	19.33 $\pm$ 0.46	19.28 $\pm$ 0.58	19.72 $\pm$ 0.55	19.92 $\pm$ 0.15
WBC ($10^3/\mu\text{L}$)	3.57 $\pm$ 0.35	3.68 $\pm$ 0.44	3.9 $\pm$ 0.45	4.66 $\pm$ 0.37 ^a	4.75 $\pm$ 0.99 ^a	3.83 $\pm$ 0.31 ^b	3.52 $\pm$ 0.20	4.39 $\pm$ 0.54 ^b
Lymphocyte (%)	76 $\pm$ 0.89	74.4 $\pm$ 2.66	75.4 $\pm$ 2.2	74.75 $\pm$ 1.92	75.25 $\pm$ 0.83	74.4 $\pm$ 1.85	73.6 $\pm$ 2.58	74.0 $\pm$ 0.82 ^a
Neutrophil (%)	20.2 $\pm$ 2.04	22.2 $\pm$ 3.37	21.2 $\pm$ 3.3	22.75 $\pm$ 3.83	20.5 $\pm$ 1.5	21.2 $\pm$ 1.47	22.8 $\pm$ 2.71	23.5 $\pm$ 1.12 ^a
Platelet ($10^3/\mu\text{L}$)	100.8 $\pm$ 7.08	107 $\pm$ 27.13	74.5 $\pm$ 18.57	76.5 $\pm$ 19.87 ^c	119.25 $\pm$ 0.99 ^c	117.6 $\pm$ 12.39 ^c	106.2 $\pm$ 8.38	111.75 $\pm$ 8.84 ^c

^a significantly different from the Control(NC); n=5

^b significantly different from that of its non-injured counterpart.

^c of significant difference when compared with LEL

Values with ^b symbol are of significant difference

PCV- Packed cell volume, WBC-White blood cell count, MCV-Mean corpuscular volume, MCH- Mean corpuscular hemoglobin; MCHC- Mean corpuscular hemoglobin concentration; RBC- Red blood cell count, Hb- Hemoglobin concentration.

The mean hemoglobin concentration values parallel those of the PCV. Although no significant difference was observed in the red blood cell (RBC) counts, the LI group had the highest mean RBC count while group HE had the least count (8.72 ± 0.12 vs $8.32 \pm 0.62 \times 10^6$). The leucocyte (white blood cell) counts of the medium extract (ME) and low dose extract with liver injury (LEL) groups were significantly elevated when compared with that of the control (4.75 ± 0.99 , 4.66 ± 0.37 vs $3.57 \pm 0.35 \times 10^3$). The high dose extract with liver injury (HEL) group had a significantly elevated WBC count than its non-injury counterpart (HE) (4.39 ± 0.54 vs $3.52 \pm 0.20 \times 10^3$). The WBC count of LEL was also observed to be markedly elevated in comparison with that of the MEL (4.66 ± 0.37 vs $3.83 \pm 0.31 \times 10^3$). In terms of differentials, the white blood cells were predominantly lymphocytes. Both the lymphocyte and neutrophil counts of the HEL group were significantly different from those of the control group (74.0 ± 0.82 vs 76 ± 0.89 ; 23.5 ± 1.12 vs 20.2 ± 2.04 %). The platelet count of group CN was similar to those groups ME, MEL, HE and HEL but higher than those of groups LE and HEL. Also, the platelet count of group LEL was significantly lower to those of the other extract groups. As for the mean corpuscular volume (MVC), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH), the results were not significantly different from those of the control or even between the groups.

DISCUSSION

The hemoglobin moiety of the red blood cell is responsible for oxygen carriage and delivery to the organs and tissue for proper metabolic function. Administration of carbon tetrachloride to induce hepatic injury triggers off an acute inflammatory response this creates a stressful milieu. As a coping strategy, there will be an increased tissue metabolism with consequent elevated oxygen demand. This will stimulate increased red blood cell production (erythropoiesis) by the stem cells. This may explain why the liver injured group of this study had the highest packed cell volume (PCV). Also, the PCVs were not significantly different amongst the groups this might be due to the durations of the acute liver insult and administration of the extracts. If these had been chronic (ie long durations) the picture might have been different.

The total circulating red cell mass may be elevated or reduced absolutely or relatively. Relative alteration known as hemoconcentration (elevation) or hemo-dilution (reduction) usually results from altered volume of circulatory body fluid. Absolute reduction in circulating red cell mass known as anemia presents clinically as low PCV and is much more common than absolute elevation which is usually due to polycythemia. Classification of anemia is premised on alterations of the red cell morphology. Such alterations include size (normocytic, microcytic and macrocytic), degree of hemoglobinization (normochromic or hypochromic) and shape. Each of these types of anemias has different aetiology, abnormal hemoglobin synthesis usually causes microcytic hypochromic anemias while macrocytic varieties result from abnormalities that impair maturation of erythroid precursors in the bone marrow. Treatment of anemia depends on the type, thus proper classification is crucial and a prerequisite for effective

therapy that will ultimately transform to an excellent prognosis. Evaluation of the red cell mass is very important for correct classification of anemia. Laboratory parameters used to evaluate red cell mass include:- the (i) Mean cell volume which is the average volume of a red cell, (ii) Mean cell hemoglobin which is the average mass of the hemoglobin content of a red cell, (iii) Mean cell hemoglobin concentration- this is the average concentration of hemoglobin in a given volume of packed red cells and (iv) Red cell width- this is the coefficient of variation of red cell volume (Notta F *et al.*, 2016). In this study, all these red cell parameters of the experimental groups were similar to those of the control and were neither affected by the chemical toxin nor by the plant extract. This might be due to the fact that erythropoiesis ex utero occurs in the bone marrow and not in the liver.

Blood cells are formed from stem cells through a series of oligopotent progenitors that progressively become unipotent. These oligopotent progenitors largely reside in the fetal liver while the unipotent progenitors that give rise to the specific three blood cell lines domicile mostly in the bone marrow (Notta F *et al.*, 2016). The induction of hepatic injury did not appear to affect leucocyte counts as the values for both control and liver injury groups were similar. However, the extract appeared to stimulate production of white blood cells (WBC) as evidenced by significant leukocytosis in groups LEL, ME and HEL. The non-injured medium extract group (ME) had higher but insignificant WBC count than the LEL and HEL. Also the leucocyte count of ME was significantly higher than that of its liver injured group (MEL). Also, the injured low and high extract groups had significant higher WBC counts than their respective non-injured groups. Thus low and high doses of *V. angustatus* extract stimulated leukocytosis while medium dose of the extract depressed it ie leukopenia. This may be due to the well-known erratic behaviour of plant extracts hence the need to exercise caution when recommending plant extracts either as supplement, therapeutic or preventive. Differential analysis of the WBC counts revealed lymphocyte predominance. Lymphocytes are largely responsible for the integrity of the immune system through synthesis of antibodies. This may be the mechanism via which *V. angustatus* exerts its immunological function. High neutrophil count in the blood known as neutrophilic leukocytosis is mostly due to acute bacterial infections (Kumar *et al.*, 2015). In this study, the HEL had a significantly higher neutrophil differential when compared with the control, this was not absolute but rather relative as the overall picture was that of lymphocyte predominance.

The liver has an excellent regenerative capability, this occurs through division of matured hepatocytes and cholangiocytes within the liver. These cells are usually mitotically dormant (Boulter *et al.*, 2013). When there is severe liver injury, the ability of the hepatocytes and cholangiocytes to regenerate may become overwhelmed. In this situation, the hepatic stem (progenitor) cells become activated (Espanol-Suner *et al.*, 2012). During liver injury, fibrogenesis is enhanced by some epithelial cells that undergo epithelial to- mesenchymal transition. However, certain mesenchymal cells can transform into epithelial cells and ultimately differentiate into either hepatocytes or cholangiocytes. This suggests that multiple cell types modulate the outcome of liver injury (Xie *et al.* 2013). These

hepatic progenitor cells are located in the canals of Hering (Theise ND *et al.*, 1999), portal area (Furuyama K *et al.*, 2011) space of Disse (Kordes C *et al.*, 2013) and central vein (Tarlow BD *et al.*, 2014). The space of Disse also contain haematopoietic stem cells that rarely divide, this may explain why haematopoiesis does not occur in the liver ex utero. In acute liver insult, the most damaged cell type is the hepatocyte and in humans, for hepatic stem cell activation there has to be minimum of 50 % loss of hepatocytes (Falkowski *et al.*, 2013; Katoonizadeh A *et al.*, 2006). Depending on the extent of the injury, the hepatic stem cell activation response may be extended. This extension is usually through the remodelling of the extracellular matrix via the production of metalloproteinases by the inflammatory cells (Fallowfield *et al.*, 2007). Thus, the diversity and plasticity of hepatic stem cells and their environment coupled with the molecular signalling determine liver homeostasis, regeneration, fibrosis and cancers under physiological and pathological conditions (Chen *et al.*, 2017). Hematopoietic stem cells are said to be observed in mice foetal liver from embryonic day 11 till day 6 post-partum after which they relocate to the bone marrow (Guo *et al.*, 2009). Thus, the liver is not a hematopoietic site in the adult rodent.

In conclusion, the ethanolic extract of *Vitex angus castus* plant causes increase in circulating white blood cell count in rats with chemical induced acute liver injury. This alteration is in a non-specific dose dependent manner.

Acute liver insult, if not adequately managed may be a precursor of chronic liver disease which more often than not carries a dismal prognosis. Though the liver is not a hematopoietic organ ex utero, the symptomatology of chronic liver disease is usually multiorgan and multisystemic. Thus the finding of this study will contribute to reduction in the incidence of chronic liver disease by ameliorating the negative effect of acute liver insult

REFERENCES

- Boulter L., Lu W.Y., Forbes S.J. (2013). Differentiation of progenitors in the liver: a matter of local choice. *J Clin Invest*, 123, 1867–1873.
- Butterworth R.F., Norenberg M.D., Felipo, V., *et al.*, (2009). Experimental models of hepatic encephalopathy: ISHEN guidelines. *Liver Int.* 29, 783–788.
- Chen J., Chen L., Zern M.A., *et al.*, (2017). The diversity and plasticity of adult hepatic progenitor cells and their niche. *Liver Int.* 2017 Sep;37(9):1260–1271.
- Cong M., Zhao, W., Liu, T., *et al.*, (2017). Protective effect of human serum amyloid P on CCl₄-induced acute liver injury in mice. *Int. J. Mol. Med.* 40, 454–464.
- Espanol-Sunier R., Carpentier R., van Hul N, *et al.*, (2012). Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. *Gastroenterology*.143:1564–1575.
- Falkowski O., An H.J., Ianus I.A., *et al.*, (2003). Regeneration of hepatocyte ‘buds’ in cirrhosis from intrabiliary stem cells. *J Hepatol.* 39:357–364.
- Fallowfield J.A., Mizuno M., Kendall T.J., *et al.*, (2007). Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J Immunol.* 178:5288–5295.
- Furuyama K., Kawaguchi Y., Akiyama H., *et al.*, (2011). Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet.* 43:34–41.
- Hajdú Z., Hohmann J., Forgo P., Martinek T., Dervarics M., Zupkó I, *et al.*, (2007). Diterpenoids and flavonoids from the fruits of *Vitex agnus-castus* and antioxidant activity of the fruit extracts and their constituents. *Phytother Res.* 21:391–4.
- Guo Y., Zhang X., Huang J., Zeng Y., Liu W., Geng C., *et al.*, (2009). Relationships between hematopoiesis and hepatogenesis in the mid trimester fetal liver characterized by dynamic transcriptomic and proteomic profiles. *PLoS One*.4(10):e7641.
- Jarry H., Spengler B., Porzel A., Schmidt J., Wuttke W., Christoffel V., (2003). Evidence for estrogen receptor beta-selective activity of *Vitex agnus-castus* and isolated flavones. *Planta Med.* 69:945–7.
- Kamal N., Mio Asni N.S., Rozlan I.N.A., Mohd Azmi M.A.H., Mazlan N.W., Mediani A., *et al.*, (2022). Traditional Medicinal Uses, Phytochemistry, Biological Properties, and Health Applications of *Vitex* sp. *Plants (Basel)*. 11(15):1944.
- Katoonizadeh A., Nevens F., Verslype C., Pirenne J., Roskams T., (2006). Liver regeneration in acute severe liver impairment: a clinicopathological correlation study. *Liver Int.* 26:1225–1233.
- Kordes C., Haussinger D., (2013). Hepatic stem cell niches. *J Clin Invest.* 123:1874–1880.
- Kumar V., Abbas A.K., Aster J.C., eds, (2015). Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia, PA: Elsevier; 583–584.
- Maiti K., Mukherjee K., Gantait A., Ahamed H.N., Saha B.P., Mukherjee P.K., (2005). Enhanced therapeutic benefit of quercetin–phospholipid complex in carbon tetrachloride-induced acute liver injury in rats: A comparative study. *Iranian Journal of Pharmacology & Therapeutics* 4:84–90.
- Notta F, Zandi S, Takayama N, *et al.*, (2016). Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. *Science* 351(6269):aab2116.
- Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats. *Journal of Pineal Research* 2000; 28(2):119–126.
- Senatore F., Napolitano F., Ozcan M., (2003). Chemical composition and antibacterial activity of essential oil from fruits of *Vitex agnus-castus* L. (Verbenaceae) growing in Turkey. *J Essential Oil-Bearing Plants*.6:185–90.
- Tarlow B.D, Pelz C., Naugler W.E., *et al.*, (2014). Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell* 15:605–618.
- Theise N.D., Saxena R., Portmann B.C., *et al.*, (1999). The canals of Hering and hepatic stem cells in humans. *Hepatology*.30:1425–1433.
- Wei Y.Y., Wang H.R., Fan Y.M., *et al.*, (2023). Acute liver injury induced by carbon tetrachloride reversal by Gandankang aqueous extracts through nuclear factor erythroid 2-related factor 2 signaling pathway. *Ecotoxicol Environ Saf.* 251:114527.
- Xie G., Diehl A.M., (2013). Evidence for and against epithelial-to-mesenchymal transition in the liver. *Am J Physiol Gastrointest Liver Physiol*.305:G881–G890.
- Zhang D.G., Zhang C., Wang, J.X., *et al.*, (2017). Obeticholic acid protects against carbon tetrachloride-induced acute liver injury and inflammation. *Toxicol. Appl. Pharmacol.* 314, 39–47.

Physiology Education

Evaluation of Peak Expiratory Flow Rate amongst Second Year Medical Students in A Nigerian University During Physiology Practical

***Ochayi O.M.¹ Akinlade B.¹, Ikemefuna N.¹, Baba Z.M.¹, Nnwanko M.²,
Madueke S.P.³**

¹Department of Physiology, Baze University, Abuja, Nigeria

²Department of Human Anatomy, Federal University Lafiya, Nassarawa State, Nigeria

³Department of Human Physiology, University of Nigeria, Enugu Campus, Enugu state, Nigeria

Summary: The present study evaluates the Peak Expiratory Flow Rate (PEFR) among second-year medical students during a physiology practical session at the College of Medicine and Health Sciences, Baze University, Abuja. The study involved 20 students (10 males and 10 females) aged 17-25 years. Anthropometric measurements, including standing height (in cm) and weight (in kg), were taken, and PEFR was measured in liters/minute using a Mini Wright Peak Flow Meter. An unpaired sample t-test was performed to compare anthropometric and physiological variables between genders. $P < 0.05$ was set at the value of statistical significant. Results indicated that PEFR was significantly higher in males than females ($P < 0.001$), independent of height, weight, and BMI. Additionally, a significant inverse correlation between PEFR and BMI was observed in males ($r = -0.67$, $P < 0.05$), whereas no significant correlation was found in females. This study underscores the gender differences in PEFR among medical students and highlights the influence of BMI on PEFR in males. These findings contribute to a better understanding of respiratory physiology in young adults and emphasize the importance of considering gender-specific factors in assessments of respiratory functions.

Keywords: Peak Expiratory Flow Rate, Medical students, Respiratory functions, Physiology.

*Authors for correspondence: mykelochayi@yahoo.com, Tel: +234- 8024662055

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INTRODUCTION

The peak expiratory flow rate (PEFR) is a crucial measure of respiratory function, which calculates the maximum gas flow expressed in litres per minute (L/min). It provides valuable insight into the pulmonary status of individuals' exhalation from a position of maximal inspiration (Clayton *et al.*, 2014). The British Thoracic Society also suggests it as key assessor for respiratory health for the management and diagnosis of asthma and chronic obstructive pulmonary disease (COPD) (Bansal *et al.*, 2012). In educational and clinical context, the understanding and application of PEFR offers significant benefits, particularly in the training of medical students who will end up as future healthcare providers (Good, 2003; Eleje *et al.*, 2024a).

The peak expiratory flow rate (PEFR) is a simple yet valuable technique that enhances the understanding of pulmonary function while highlighting the variability and clinical significance of respiratory measurements (Kaufman, 2018; Mann, 2011). Over the years, research has documented variations in PEFR across physiological and pathological factors, including well-established sex differences in respiratory parameters. However, regional influences, particularly in tropical climates, require further

exploration to determine if these variations align with findings from other climatic regions.

Medical education integrates physiological practical sessions to provide hands-on experience and reinforce theoretical knowledge. Among these, respiratory physiology experiments are essential, enabling students to measure and analyze their own respiratory parameters, such as PEFR (Berkhout *et al.*, 2018; Eleje *et al.*, 2024b). This experiential learning approach effectively prepares medical students for clinical practice while offering a valuable opportunity to investigate gender-related differences in respiratory function.

Despite the importance, time and effort of these practical sessions, there are paucity in the documentation of data obtained from PEFR among medical students during practical classes, considering most of the reference values we use in Nigeria were obtained from US soldiers which could have differ from that of tropical climes like ours. As study conducted by Hakim *et al.*, (2023), shows that the documentation of scientific evidence aids the integration of medical science and clinical practises. Hence, this study will be valuable for several reasons. It may serve to establish the normal range of PEFR values in a specific demography, and aids in the calibration of instructional tools and set-points

for medical education and geographical variance. As well as serve as a baseline for comparative studies involving different interventions or approaches aimed towards improving the respiratory health of a given society.

This study evaluates the peak expiratory flow rate among second year medical students during physiological practical sessions by exploring the potential correlations between PEFR and variables such as gender and body mass index (BMI).

MATERIALS AND METHODS

This cross-sectional study involved second-year medical students at Baze University's College of Medicine and Health Sciences during their physiology practical session. Participation was voluntary, as is customary for reinforcing theoretical knowledge after a 30-minute pre-lab demonstration. The study followed the Department of Human Physiology's Laboratory Manual (Exp. 25, Pp. 90) and adhered to ethical guidelines for human subject research, as approved by the Department. Out of 30 students, 10 were excluded based on health and age, leaving a sample of 20. Due to the limited population, Cochran's formula was inapplicable, and the Krejcie & Morgan table was used, incorporating the entire eligible population. The final sample consisted of 10 males and 10 females, aged 17 to 25 years.

Experimental Protocol: On the experimental day, a 30-minute pre-lab talk and demonstration were conducted to educate students on the significance of the experiment. The inclusion criteria required participants to be second-year medical students at Baze University, Abuja, aged between 17 and 25 years at the time of the experiment. Students with diagnosed respiratory or cardiovascular diseases were excluded.

Informed consent was obtained from each participant before they were seated in a relaxed condition. The standing height (cm) of each participant, measured without shoes, and their weight (kg) were recorded using a stadiometer. The peak expiratory flow rate (PEFR) was measured in liters per minute using a Mini Wright Peak Flow Meter. The procedure was as follows: participants were seated, and a nose clip was applied to prevent nasal airflow. The mouthpiece of the peak flow meter was placed in the participant's mouth, held firmly between the teeth, and sealed with the lips. After a maximal inspiratory effort, participants exhaled with maximum force. The PEFR was measured three times, and the highest value was recorded as the accepted value, following the guidelines of the American Thoracic Society (ATS) (Babu et al., 2015).

Statistical Analysis: Descriptive analyses were expressed in terms of mean±standard deviations (SDs). Normality of data distributions was analyzed using the Shapiro-Wilk test and boxplot. Unpaired sample t-test was used to test for significant difference at P<0.05.

RESULTS

The Peak expiratory flow rate was significantly higher in males than in females (P-value < 0.001). The statistically insignificant difference (P > 0.05) in anthropometric variables indicates that peak expiratory flow rate is significantly higher than the differences in both males and females is independent of height, weight and BMI (Table 1). There was a strong linear correlation between weight and BMI. Intriguingly, peak expiratory flow rate showed statistically significant inverse association with BMI with a correlation coefficient of -0.67 (P <0.05) in males but no statistically significant association with females (Table 2).

DISCUSSION

In this study, we explored the anthropometric and physiological differences between males and females, with a specific focus on the Peak Expiratory Flow Rate (PEFR). The PEFR significantly increases in males compared to females which is in agreement with existing physiology literatures indicating that males generally have higher lung volumes and respiratory capacities than females, which can be attributed to anatomical and physiological differences such as larger airway diameters and greater lung volumes in males (Mishra et al, 2013, Babu et al., 2015, Jena et al., 2017). However, there were no significant differences in anthropometric variables such as height, weight, and BMI between the sexes. This finding contradicts some literature that has reported sex-based differences (Sanchez et al., 2018). The results of this study may be due to a relatively small sample size or similar physical characteristics among the participants (Aliasghari et al., 2020).

Previous research has shown that while there are average differences in height and weight between males and females, these differences can vary significantly based on the population and specific sample characteristics (Danborno et al., 2021; Cattelino et al., 2025). As expected, weight and BMI showed a strong positive linear correlation in both males and females as BMI is a direct function of weight and height. One intriguing finding is the significant inverse association between PEFR and BMI in males; this suggests that higher BMI is associated with lower PEFR in males, which aligns with studies indicating that increased adiposity can negatively impact pulmonary function due to factors like reduced chest wall compliance and respiratory muscle inefficiency (Severin, 2022).

Table 1: Selected Anthropometric variable and peak expiratory flow rate

	Male	Female		
Variables	Mean ± SD	Mean ± SD	t-value	P-value
Height (cm)	178.80 ± 5.90	174.20 ± 4.26	2.00	0.061
Weight (kg)	92.20 ± 14.85	80.90 ± 16.53	1.61	0.125
BMI (kg/m²)	28.85 ± 4.37	26.66 ± 5.38	1.00	0.329
PEFR (L/min)	556.30 ± 89.59	402.40 ± 71.86	4.24	<0.001

Table 2:

Correlation matrix of the key variables

Variables	1	2	3	4
1. Height	1	0.29	-0.12	0.34
2. Weight	0.24	1	0.92**	-0.53
3. BMI	-0.01	0.97**	1	-0.67*
4. PEFR	0.57	0.58	0.45	1

Above the diagonal, males; below the diagonal, females

**: Correlation is significant at the 0.01 level (2-tailed)

*: Correlation is significant at the 0.05 level (2-tailed)

Interestingly, this association was not observed in females, which might be due to differences in fat distribution patterns, hormonal influences, or other sex-specific physiological factors (Cyril *et al.*, 2024). These findings have important implications for understanding sex differences in respiratory health and the impact of body composition on lung function (Babu *et al.*, 2015, Jena *et al.*, 2017). The significant inverse correlation between PEFR and BMI in males highlights the potential respiratory risks associated with higher BMI, emphasizing the need for targeted interventions to address obesity and its impact on respiratory health in men.

Future research should aim to explore the underlying mechanisms driving the sex-specific differences observed in this study. Larger, more diverse samples could provide a clearer picture of these relationships and help determine whether these findings are generalizable across different populations. Additionally, longitudinal studies could investigate how changes in BMI over time affect PEFR and whether similar patterns are observed in females with larger sample sizes.

In conclusion, this study further highlights the importance of considering sex differences in respiratory health research. The significant difference in PEFR between males and females, independent of anthropometric variables, along with the inverse relationship between PEFR and BMI in males observed in this study, highlights the complex interplay between sex, body composition, and pulmonary function. These findings contribute to the broader understanding of respiratory physiology and can inform clinical practices and public health strategies aimed at improving respiratory health outcomes.

REFERENCES

Aliasghari, F., Asghari Jafarabadi, M., Lotfi Yaghin, N., & Mahdavi, R. (2020). Psychometric properties of Power of Food Scale in Iranian adult population: Gender-related differences in hedonic hunger. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity*, 25(1), 185–193.

Babu, K. R., Mohit, M., & Kolekar, D. (2015). A comparative study of peak expiratory flow rate (PEFR) and BMI in male and female medical students. *International Journal of Health Sciences and Research*, 5(1), 273–278.

Bansal, M., Goyal, M., Dhillon, J. K., & Kaur, P. (2012). Longitudinal study of peak expiratory flow rate in pregnant women. *National Journal of Integrated Research in Medicine*, 3(1), 34–38.

Berkhout, J. J., Helmich, E., Teunissen, P. W., van der Vleuten, C. P., & Jaarsma, A. D. C. (2018). Context matters when striving to promote active and lifelong learning in medical education. *Medical Education*, 52(1), 34–44. <https://doi.org/10.1111/medu.13456>

Cattellino, E., Bina, M., Skanjeti, A. M., & Calandri, E. (2015). Anthropometric characteristics of primary school-aged children: Accuracy of perception and differences by gender, age, and BMI. *Child: Care, Health and Development*, 41(6), 1098–1105. <https://doi.org/10.1111/cch.12272>

Clayton, T. C., Meade, T. W., Turner, E. L., & De Stavola, B. L. (2014). Peak flow rate and death due to coronary heart disease: 30-year results from the Northwick Park Heart cohort study. *Open Heart*, 1(1), e000164.

Cyril, J. M., Sowmya, R., & Viswanatha, B. (2024). A cross-sectional study of pulmonary impairment pattern in adults with central obesity. *International Medicine*, 10(1). <https://doi.org/10.2667-7008>

Danborno, A. M., Ogbe, S. O., Mallo, M. J., Toryila, J. E., Shafe, M. M. O., Ochayi, M. O., & Danborno, B. (2021). Knowledge, Attitude, Practice and Stigma Related to COVID-19: A Nigerian Perspective. *Nigerian Journal of Physiological Sciences*, 36(1), 17–24.

Eleje, G. U., Ikwuka, D. C., Nwosu, K. C., Eleje, L. I., Sani, N., Niyoyita, J. P., ... & Nwobodo, E. O. (2024). Improving struggling medical and nursing students' subjective vitality, school engagement and academic performance through a peer mentorship intervention programme: an intervention protocol. *BMC Medical Education*, 24(1), 1144.

Eleje, G. U., Ikwuka, D. C., Nwosu, K. C., Eleje, L. I., Sani, N., Niyoyita, J. P., ... & Nwobodo, E. O. (2024). Exploring the Positive Feeling of Physical and Mental Energy among Struggling Preclinical Medical Students in Nigeria: A pilot Observational Study. *International Journal of Research and Innovation in Social Science*, 8(3s), 4610–4622.

Good, M. L. (2003). Patient simulation for training basic and advanced clinical skills. *Medical Education*, 37(1), 14–21. <https://doi.org/10.1046/j.1365-2923.2003.01457.x>

Hakim, A., Ricketts, W., Pfeffer, P., & Ashworth, R. (2023). Integration of respiratory physiology and clinical reasoning in the early years of a medical curriculum: Engaging with students in a large classroom setting. *Advances in Physiology Education*. <https://doi.org/10.1152/advan.00042.2023>

Jena, S. K., Mirdha, M., Meher, P., & Misra, A. K. (2017). Relation of peak expiratory flow rate to body mass index in young adults. *Muller Journal of Medical Sciences and Research*, 8(1), 19–23.

Kaufman, D. M. (2018). Teaching and learning in medical education: How theory can inform practice. In T. Swanwick, K. Forrest, & B. C. O'Brien (Eds.), *Understanding medical education: Evidence, theory, and practice* (pp. 37–69). Wiley-Blackwell. <https://doi.org/10.1002/9781119373780>

Mann, K. V. (2011). Theoretical perspectives in medical education: Past experience and future possibilities. *Medical Education*, 45(1), 60–68.

Mishra, J., Mishra, S., Satpathy, S., Manjareeka, M., Nayak, P. K., & Mohanty, P. (2013). Variations in PEFR among males and females with respect to anthropometric parameters. *IOSR Journal of Dental and Medical Sciences*, 5(1), 47–50.

Physiology Laboratory Manual. (2022). Experiment 25. Baze University, Abuja, Nigeria p. 90.

Sanchez, F. F., Araújo da Silva, C. D., de Souza Pereira Gama Maciel, M. C., Rebouças Demóstenes Marques, J., Brosina de Leon, E., & Lins Gonçalves, R. (2018). Predictive equations for respiratory muscle strength by anthropometric variables. *The Clinical Respiratory Journal*, 12(7), 2292–2299. <https://doi.org/10.1111/crj.12950>

Severin, R. S. (2022). The effects of respiratory muscle performance on clinical outcomes in obese patients [Doctoral dissertation, University of Illinois at Chicago].