Niger. J. Physiol. Sci. 35 (June 2020): 115 - 116
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Tribute

Profile of a Don - Prof. Eme Osim Retires

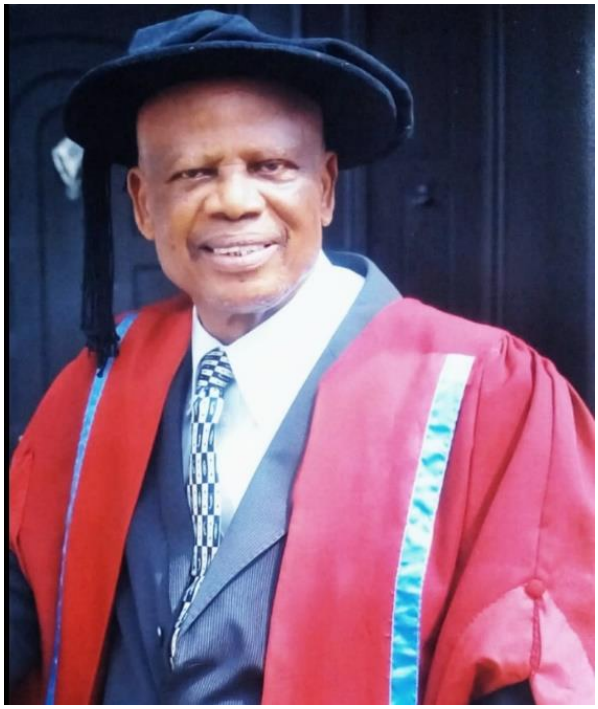
Daniel U. Owu

Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Nigeria

Summary: Prof Eme E. Osim served Editor-in-Chief of Nigerian Journal of Physiological Sciences between 1999 and 2009. This tribute highlights his immense contributions to the development of the journal and academia.

Keywords*: Tribute, Eme Osim, Retirement, Nigerian Journal of Physiological Sciences*

**Address for correspondence*: *d_owu@yahoo.com Tel: +234 8061650527*



Prof. Eme E. Osim
Editor-in-Chief (1999 – 2009)

The month of November 2020 marked the end of an era: the retirement after 43 years and four months of Prof. Eme E. Osim at the University of Calabar. As a former Editor-in-Chief of our prestigious journal, his services to the journal, an official publication of the Physiological Society of Nigeria is ranked as one of the most successful of an erudite scholar and researcher in Nigeria. During his years as the Editor-in-Chief, the Nigerian Journal of Physiological Sciences was indexed by many abstracting bodies such as National Library of Medicine (PubMed), Bioline, African Journals Online (AJOL) and Index Copernicus to mention but a few. He served the scientific community with dedication, passion and diligence.

Professor Eme Efiom Osim was born on 28th November, 1950 in Usumutong, Abi Local Government Area of Cross River State, Nigeria. He completed his primary education at the Presbyterian Primary School, Abakaliki in 1962 and secondary education at the prestigious Hope Waddell Training Institute, Calabar, Cross River State.Eme Efiom Osim got admitted into the foremost University in Nigeria, University of Ibadan, Nigeria in 1972 and completed his B.Sc in Human Physiology in 1976. After his graduation, Eme Osim served his country under the mandatory National Youth Service Corp (NYSC) programme during the 1976/1977 NYSC year at School of Nursing, Sokoto. He belonged to the elite group of Nigerians who had his M.Sc degree in 1979 and a doctoral degree in Physiology at the famous University College, London in England. His research interest is in Neuro-physiology and Gastrointestinal Physiology. In the first year of his Ph.D. programme, he won Wellcome Trust Sponsorship and became a Wellcome Trust scholar in addition to being a University of Calabar scholar. He rose through the ranks to become Professor of Physiology in 1998. Professor Osim is one of the eleven physiologists in Nigeria awarded Fellow of Physiological Society of Nigeria (FPSN) for meritorious services to the growth of Physiology in Nigeria. Prof Osim was again awarded the Fellowship of Nigerian Society for Experimental Biologists (FNISEB) in 2016. He has also benefited from TETFUND Institution based research grant in 2015/2016. He served as Associate Professor and Professor of Physiology in the Universities of Zimbabwe and Malawi respectively between 1997 and 1999.

After gaining research and teaching experience within and outside Nigeria, he settled down in University of Calabar, Calabar, Nigeria where he contributed immensely to the growth and development of human resources within the university and its environment. He was the Head, Department of Physiology for over 13 years. In 1998, he was

appointed Professor of Physiology at University of Calabar. He was the Deputy Provost, College of Medical Sciences from 1994-1996, member of University of Calabar Governing Council (1990 – 1993 and 2005 – 2007). He was a Sessional Consultant University of Calabar Teaching Hospital (UCTH) between October 1999 and December 2020. He was the Dean, Faculty of Basic Medical Sciences between 2004 -2008 and later became Dean of Basic Medical Sciences and Head of Okuku Campus of Cross River University of Technology (CRUTECH) from April, 2013 – March, 2014. Prof. Osim has served as the Director, Intellectual Property and Technology Transfer Office (IPTTO) in University of Calabar since April 2018 till 2021.

Prof Osim has researched on many trending issues related to our environment and health and has published over 180 research articles and four books. One hundred of these publications can be found in selective SCOPUS. He has also trained many young scientists and supervised over 48 doctoral theses. Professor Eme E. Osim was in 2018 (jointly with Dr. S.A. Bisong) awarded a patent by the Federal Republic of Nigeria for the sole use and advantage of an invention for; Method of aqueous root bark extract of Rauwolfia vomitoria for ameliorating dementia in a mouse model of Alzheimer's disease.

Prof. Osim has been a strong member of the Physiological Society of Nigeria. He has demonstrated his commitment to the Society by contributing to the growth of the Society and attending many meetings of the Society. He served the Society as the National Secretary from 1992 to 1994 and became the

Editor-In-Chief, Nigerian Journal of Physiological Sciences between 1999 to 2009. In 2011, soon after Prof Osim completed his ten-year tenure as Editor-in-chief, TETFUND chose Nigerian Journal of Physiological Sciences as one of the best 50 journals in the country and awarded a 5 million Naira grant. Prof. Osim became the President of the Physiological Society of Nigeria from 2009 to 2014. In his time as the President of the Society, many innovations concerning meetings and scientific sessions were introduced. He was appointed a Fellow of the Society in 2010 and he is currently Chairman, Board of Trustees of Physiological Society of Nigeria.

His contributions to meetings and suggestions to the Physiological Society will still be reminiscent though he bows out of the University of Calabar. He has been a mentor to young physiologists, an honest reviewer of many manuscripts, and excellent leader in research and administration. Prof. Osim has been a critical thinker and provides innovative ideas when there seems to be no way forward in research direction. The Editorial Board of the journal will continue to remember his contributions in terms of his research impact and teaching career that has produced many professors, researchers and quality research findings to the journal. As a happy family man, we congratulate Prof Osim on his retirement from active service of the University of Calabar and wish him a healthy and happy life.

Niger. J. Physiol. Sci. 35 (December 2020): 117 - 121
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

SARS-CoV-2 Infection Screening Using Two Serological Testing Methods

***Arinola O.G[1], Edem V.F[1], Rahamon S.K.[1], Yaqub S.A[1], Fashina A.O.[2] and Alonge T.O.[3,4]**

[1]*Department of Immunology, College of Medicine, University of Ibadan., Ibadan, Nigeria*
[2]*Department of Medical Microbiology and Parasitology, University College Hospital, lbadan. Ibadan, Nigeria*
[3]*lnfectious Diseases Centre, Olodo, lbadan, Nigeria*
[4]*Department of Surgery, College of Medicine, University of Ibadan. Ibadan, Nigeria*

Summary: The challenges associated with adequate deployment of nucleic acid amplification tests (NAATs) in developing countries underscores the important role of simple but sensitive and specific serological testing kits in COVID-19 diagnosis. Presently, there are a number of point-of-care tests for Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) screening. However, the reliability of these test kits is poorly documented and hence, needs to be ascertained. This study was therefore designed to determine the sensitivity and specificity of two serological test kits for COVID-19 screening with the view to providing necessary information on the suitability of their deployment as routine test kits for SARS-CoV-2 in Nigeria. Forty-seven (47) asymptomatic adults who had been tested for SARS-CoV-2 with the real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) were enrolled into this study. Blood samples were obtained for qualitative determination of serum IgM and IgG antibodies to the S-antigen of SARS-CoV-2 using a commercially available IgM and IgG Rapid Diagnostic Test (RDT) and enzyme linked immunosorbent assay (ELISA). The association between the test kits (ELISA and RDT) and PCR in diagnosing COVID-19 was determined using the Fisher's Exact test at $P<0.05$. 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 anti- SARS COV-2 specific antibodies. 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 anti-SARS-CoV-2 IgM antibody identified using ELISA was significantly higher compared with RDT. In contrast, the proportion of participants with positive anti- SARS COV-2 IgG antibody identified using RDT was significantly higher compared with ELISA. ELISA has a better sensitivity for detecting anti-SARS-CoV-2 Spike-protein specific antibodies than the RDT. However, combination of RDT and ELISA for the detection of anti-SARS-COV-2 antibodies might be useful for population COVID-19 screening

Keywords*:* Anti-SARS-CoV-2 antibody, COVID-19 screening, ELISA, Rapid diagnostic strip, Serology

**Address for correspondence*: drarinolaog64@yahoo.com, *Tel: +234-8023451520*

Received- November, 2020; Accepted- December, 2020

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a pandemic which continues to highlight the need for different types of validated diagnostics tests with a view to meeting global demands (Weissleder *et al*., 2020). This demand is more pronounced in developing countries where availability and accessibility to molecular testing could be limited.

To provide epidemiologic data and to contain outbreaks, a number of diagnostic testing methods and kits have been developed for COVID-19 with each method performing distinct roles. These include the nucleic acid, serological, antigen, and ancillary tests (FDA 2020; Weissleder *et al*., 2020; Yang *et al*., 2020) The nucleic acid amplification tests (NAATs) are the most widely used and recommended test for the diagnosis of SARS-CoV-2, identification of strains or mutations and triage of patients. NATs detect the presence of the viral genome and indicate an active, current infection but cannot detect a prior infection including recently resolved ones (Jacofsky *et al*., 2020).

Another important testing method is the serological test. This test detects the anti-SARS-CoV-2 antibodies (mainly IgG, IgM and IgA) produced by the immune system. This test is not indicated for early diagnosis and screening for active early infection of COVID-19 as there is a time lag (6 – 7 days) for immune response to the antigenic viral invasion

culminating in SARS-CoV-2 specific antibody production. However, essential information on the immune status, prior infection and the status of a given population related to their ability to contract or resist infection is based on the antibody status (Jacofsky *et al*., 2020).

The challenges associated with adequate deployment of NAATs in developing countries including Nigeria underscores the important role of cost-effective, simple but sensitive and specific serological testing kits in COVID-19 diagnosis. This will facilitate reduction in the pressure on NATs and hasten clinical decision making. In addition, the fund saved from the procurement of NAATs equipment can be channelled towards procuring these relatively cheap serological test kits with better turn-around-time.

Presently, there are a number of point-of-care tests for SARS-CoV-2 diagnosis however, the reliability of these test kits is poorly documented and hence, needs to be ascertained. This study was therefore, designed to determine the sensitivity and specificity of two serological test kits for COVID-19 diagnosis with the view to providing necessary information on the suitability of their deployment as routine test kits for SARS-CoV-2 in Nigeria..

MATERIALS AND METHODS

Study Population: Forty seven (47) SARS-CoV-2-asymptomatic adults were enrolled into this study from The Infectious Diseases Centre, Olodo, Ibadan, Oyo State, Nigeria. The real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay was used to confirm the status of all the study participants using nasal and pharyngeal swab specimens following WHO guideline (WHO 2020b)

Sample collection: Five millilitres (5 ml) of venous blood was obtained from each study participant and was dispensed into plain sample bottles to obtain sera as appropriate. Participants with positive result for PCR assay were followed up till discharge and 5 ml of venous blood was also obtained at the point of discharge. An aliquot of each serum was used for the RDT while the remaining portion of the serum was stored at -20^{0}C for ELISA analysis.

Ethical consideration: The study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/20/0233) and informed consent was obtained from each study participant.

Laboratory analyses: Qualitative determination of IgM and IgG antibodies to the Spike-protein of SARS-CoV-2 was done using two methods (ELISA and Rapid Diagnostic Test) following the manufacturers' instruction. Briefly, the ELISA kits qualitatively detect human IgM and IgG antibodies specific for SARS-C.

The specific antibodies in the sample bind with the SARS-CoV-2 spike-protein antigen in pre-coated wells of microtitre plates. Thereafter, horseradish peroxidase (HRP) conjugated mouse anti human IgG and IgM respectively is added to the wells to form antigen-antibody-HRP conjugated secondary antibody complex, which is amplified using chromogen/substrate. Absorbance at 450 nm is measured and compared with the kits stipulated cut-off values to determine if a sample contains SARS-CoV-2 spike-protein specific IgM or IgG. Samples with absorbance value equal to or above the cut-off values are regarded as positive for anti-SARS-CoV-2 spike-protein specific IgM or IgG antibodies while samples with absorbance value less than the cut-off are regarded as negative for SARS-CoV-2 spike-protein specific IgM or IgG antibodies. For RDT, the sample reacts with SARS-CoV-2 S-antigen coated on the conjugate pad. If specific antibodies are present in the sample, the anti- SARS-CoV-2 IgM and/or IgG antibody (of the S-antigen - anti-SARS-CoV-2 IgM/IgG antibody) binds to mouse anti-human monoclonal antibodies on the IgM and IgG lines. The presence of anti-SARS-CoV-2 IgM and/or IgG is indicated by a visible test line on the IgM and IgG lines in addition to the visible control line.

Estimation of diagnostic performances*:* The sensitivity and specificity of the test kits were determined using ROC. Thereafter, 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 earlier described (Molinaro 2015; Trevethan 2017).

Statistical Analysis

Statistical analysis was carried out using the SPSS statistical software version 23.0 for windows. The association between the test kits (ELISA and RDT) and PCR in diagnosing COVID-19 was determined using the Fisher's Exact test. *P*-value less than 0.05 was considered as statistically significant.

RESULTS

The presence of SARS-CoV-2 based on the 3 methods is presented in Table 1. 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 anti- SARS-CoV-2 specific antibodies (Table 1).

In order to understand the specific class of antibody detected by the ELISA and RDT, the results observed in Table 1 were stratified. As shown in Table 2, the proportion of participants with anti-SARS-CoV-2 IgM antibody identified using ELISA was significantly higher compared with RDT. In contrast, the proportion of participants with positive anti-SARS-CoV-2 IgG antibody identified using RDT was significantly higher compared with ELISA. ELISA showed that all the 47 participants had no anti-SARS-CoV-2 IgG antibody as at the point of sample collection but the RDT showed that 25.5% of the study participants had anti-SARS-CoV-2 IgG IgG antibody at diagnosis.

Table 1:
Diagnosis of COVID-19 using three different methods

	PCR		ELISA		RDT	
	Yes	No	Yes	No	Yes	No
At enrolment (n = 47)	28	19	20	27	13	34

Table 2:
Proportion of study participants with positive and negative SARS-CoV-2 IgM and IgG antibodies using ELISA and RDT

Antibodies	**ELISA**	**RDT**	χ^2	***P*-value**
IgM				
Positive	20 (42.6%)	1 (2.1%)	22.136	0.000*
Negative	27 (57.4%)	46 (97.9%)		
IgG				
Positive	0 (0.0%)	12 (25.5%)	13.75	0.000*
Negative	47 (100.0%)	35 (74.5%)		

*Significant at $P<0.05$

Table 3:
Summary of comparative diagnostic performances of ELISA and RDT in participants screened for SARS-CoV-2 infection

Indices of Diagnostic Performance	**IgM ELISA**	**IgM RDT**	**IgG RDT**
Sensitivity	64.3%	3.6%	42.9%
Specificity	89.5%	100.0%	100.0%
Positive Predictive Value	90.0%	100.0%	100.0%
Negative Predictive Value	63.0%	41.3%	54.3%
Positive Likelihood Ratio	6.12	0.036	0.0
Negative Likelihood Ratio	0.40	0.964	0.571
Diagnostic Odds Ratio	15.3	0.037	0.0
Accuracy	0.74	0.426	0.7

Comparative diagnostic performances of ELISA and RDT in participants screened for SARS-CoV-2 infection are shown in Table 3. ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT while the RDT had a better specificity performance than the ELISA. The RDT had 100% specificity as it had negative reaction for all the 17 study participants with negative PCR results. Of these 17 participants however, 2 had positive ELISA reaction hence; the specificity of ELISA was lower (89.5%) compared with the RDT.

Dynamics of IgM and IgG changes at enrolment and at discharge are shown in Table 4. The ELISA result alone was considered as no participant was found to have IgG at enrolment. It was observed that 15 participants who had anti- SARS-CoV-2 IgM antibody at diagnosis still sustained it till discharge, 5 participants who had anti-SARS-CoV-2 IgG antibody had it at discharge, 5 participants have SARS-CoV-2 IgM at diagnosis and at discharge, 3 participants with anti-SARS-CoV-2 IgM antibody at diagnosis did not have at discharge while 6 participants had anti-SARS-CoV-2 IgM antibody at diagnosis and anti-SARS-CoV-2 IgG antibody at discharge.

Table 4:
Dynamics of changes in IgM and IgG at enrolment and at discharge (using ELISA)

	Pattern of result	**Number of patients**
IgM		
At diagnosis	At discharge	
+ve	+ve	15
-ve	+ve	5
+ve	-ve	3
-ve	-ve	5
IgG		
At diagnosis -ve	At discharge +ve	6

+ve = Positive, -ve = Negative

DISCUSSION

Viral RNA detection using nucleic acid amplification test (NAAT) such as RT-PCR is the recommended test for SARS-CoV-2 infection diagnosis (La Marca *et al*., 2020). However, the long turn-around-time (TAT), owing to the numerous logistics involved in NAAT (Weissleder *et al*., 2020) as well as the poor infrastructure and resources in developing countries clearly underscores the relevance of rapid serological testing kits, which are used for binary qualitative

results determination, in revealing the factual epidemiology of COVID-19.

Relative to PCR, ELISA and RDT accurately detected 71.4% and 46.4% respectively of the study participants with SARS-CoV-2 infection. This observation indicates that the SARS-CoV-2 detection capacity of ELISA is more than that of RDT and could therefore be deployed in poor resource settings even in asymptomatic individuals. Generally, it is believed that the accuracy of serological tests improves (can be near 100%) when samples are obtained 20 days after infection when the immune response would have fully evolved (Weissleder *et al*., 2020).

SARS-CoV-2 is one of the seven coronaviruses known to have infected humans hence; issues of cross-reactivity in diagnostic tests cannot be overemphasized. In fact, false-negative results using RT-PCR due to variability in viral load and sampling have been reported in ~30% of COVID-19 patients and are thus of great concern in NAAT (Ai *et al*., 2020; Tang *et al*., 2020; Yang *et al*., 2020). Therefore, evaluation of serological testing kits performance, is critical in ensuring true identification, solely from among people who are known to have SARS-CoV-2 infection, all those who do indeed have SARS-CoV-2 infection (i.e. true positives), and avoiding categorization of other people as not having SARS-CoV-2 infection when in fact they do have it (i.e. false negatives) (Pereira 2016; Trevethan 2017). In this study, ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT. This observation supports the earlier observed higher proportion of COVID-19 patients detected by ELISA compared with the RDT. In contrast, the RDT had a higher specificity compared with ELISA. The RDT had negative reaction for all the 17 participants with negative PCR results but ELISA had negative reaction for 15 of the 17 participants. The seemingly false-positive result observed in ELISA cannot be substantiated as false-negativity is also a concern in NAAT (Tang *et al*., 2020). Zhao *et al*. (2020) reported that the sensitivity of antibody based test kits, which was lower (38.3% vs 66.7%) at the early phase of SARS-CoV-2 infection overtook that of NAAT and reached over 90% 12 days after onset of illness.

Diagnostic accuracy of antibody tests depends on its purpose and its interpretation varies. Diagnosing SARS-CoV-2 infection in symptomatic patients requires that the test has sensitivity as high as 90% with a slight reduction in specificity acceptable. In contrast, high specificity as high as 98%, is essential when antibody tests are to be used in the determination of when to terminate social isolation (La Marca *et al*., 2020). In this study, 12 out of the 13 participants with positive RDT result had IgG while only one had IgM. This observation highlights possible challenges associated with the use of RDT for SARS-CoV-2 infection screening using IgM antibody. Our observation corroborates the report of Zhang *et al*.,)2020) which showed that some patients tested with RDT were more positive for IgG than IgM at the moment of hospitalization and 5 days later; but had an earlier IgG than IgM seroconversion.

Although RDT has a better specificity compared with ELISA in this study, its poor sensitivity (3.6%) shows that ELISA has a better SARS-CoV-2 infection detection ability than RDT. The RDT even detected IgG in 25.5% of the participants who were found to be IgG-negative using ELISA. This observed discrepancy could suggest cross-reaction of the S-protein on RDT with other antigens, probably other human coronaviruses, aside SARS-CoV-2 S-protein. This suggestion could be alluded to by the observed higher sensitivity of ELISA compared with the RDT. However, the observed high specificity might suggest that RDT could be useful in making clinical decision on imunnoprotection status and when to return to normal activities following social isolation (La Marca *et al*., 2020) as all the participants with negative PCR result were equally detected as SARS-CoV-2 S-antigen specific IgM and IgG free. Due to the observed better sensitivity of ELISA over RDT and the better specificity of RDT over ELISA, it could be suggested that a combination of RDT and ELISA for SARS-COV-2 antibodies might be useful for population screening. It was suggested in the reports of Zhao *et al*. (2020) and Özçürümez *et al*. (2020) that a combination of molecular and serological tests could facilitate accurate diagnosis of patients with COVID-19 at different stages of the disease.

Diagnostic accuracy of serological testing kits is dependent on the evolvement of immune response to the immunogen of interest (La Marca *et al*., 2020). Generally, in response to infections or immunization, IgM antibodies are elicited first but disappear after a few weeks. However, IgG antibodies are produced at the same time or 2 to 3 days later and titres usually remain high for months or years (Racine and Winslow 2009; WHO 2020a).

The immune response dictates the seroconversion time and varies in COVID-19 patients. Zhao *et al*. (2020) reported median seroconversion rates of 93.1%, 82.7% and 64.7% for total antibody, IgM and IgG at 11, 12 and 14 days respectively in COVID-19 patients. Wölfel *et al*., 2020) reported that 50% of their studied patients had seroconversion after 7 days while all the patients seroconverted after 14 days. Similarly, IgG seroconversion was observed in 285 COVID-19 patients within 19 days of onset of symptoms (Long *et al*., 2020). In this study, it was observed that the pattern of IgM and IgG antibody response varies and this variation determines the outcome of serological testing. All (20) the participants with positive IgM antibody result had negative IgG antibody result when

tested using ELISA. This observation is in line with the asymptomatic status of the enrolled participants. The report of Liu *et al*., 2020) showed that anti-SARS-CoV-2 S-specific IgM and IgG antibodies were not detectable from days 0 to 3. However, specific IgM antibodies became detectable at day 4 and peaked at about day 20 before gradual decline which became marked 4 weeks after onset of symptoms. They also showed that specific IgG antibodies were detectable from day 7 and peaked at about day 25 and remained high in concentration even, after 4 weeks of infection. It has been reported that the sensitivities of antibodies for SARS-CoV-2 specific antigens increase with the increasing number of days of illness. Zhao *et al*. (2020) showed that patients in the latter phase (days 15 – 39 post infection) had 100.0%, 94.3% and 79.8% sensitivities for total antibody, IgM and IgG respectively as against 45.5% sensitivity of NAAT. The pattern of changes of antibodies observed in this study further substantiate the dynamics involved in the interpretation of anti-SARS-CoV-2 antibody test results as individual humoral immune responses against SARS-CoV-2 infection may slightly differ. Usually, positive antibody result to SARS-CoV-2 antigen(s) indicates past infection with SARS-CoV-2 which can be recent infection (concurrence of IgM and IgG) or an infection of more than a few weeks ago (IgG only). In contrast, negative antibody result to SARS-CoV-2 connotes no infection, recent infection in the last 14 days or infection with antibody levels below the level of detection of the immunoassay (WHO 2020a). The technicalities involved in the interpretation of antibody detection kits due to the dynamics of individual immune responses to infections further situates NAAT as the cornerstone diagnostic assay for SARS-CoV-2 infection. Furthermore, Wang *et al*. (2020) reported that there exist a small proportion of patients with difficulty in rapidly gaining immunity against SARS-CoV-2 even up to 50 days after their symptoms onset. It is apparent therefore, that care needs to be taken when using anti-SARS-CoV-2 antibodies for COVID-19 clinical diagnosis and determination of discharge criteria.
Small sample size was a limitation in this study.

It could be concluded from this study that ELISA has a better sensitivity for the detection of anti-SARS-COV-2 S-antigen specific antibodies than the RDT. However, combination of RDT and ELISA for the detection of anti-SARS-COV-2 antibodies might be useful for population COVID-19 screening. A large population study is still required to confirm the observations in this study before anti-SARS-CoV-2 S-antigen specific antibodies ELISA can be deployed as routine test kits for SARS-CoV-2 diagnosis and monitoring in Nigeria.

.

REFERENCES

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., Tao, Q., Sun, Z., and Xia, L. (2020). 'Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases', Radiology, 296: E32-e40.

FDA. 2020. 'Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency (2020). Available at: https://www.fda.gov/regulatory-information/search-fdaguidance-documents/policy-coronavirus-disease-2019tests-during-public-health-emergency-revised. Accessed: 08 December, 2020.

Jacofsky, D., Jacofsky, E. M., and Jacofsky, M. (2020). 'Understanding Antibody Testing for COVID-19', J Arthroplasty, 35: S74-s81.

La Marca, A., Capuzzo, M., Paglia, T., Roli, L., Trenti, T., and Nelson, S. M. (2020). 'Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays', Reproductive biomedicine online.

Liu, X., Wang, J., Xu, X., Liao, G., Chen, Y., and Hu, C. H. (2020). 'Patterns of IgG and IgM antibody response in COVID-19 patients', Emerg Microbes Infect, 9: 1269-1274.

Long, Q. X., Liu, B. Z., Deng, H. J., Wu, G. C., Deng, K., Chen, Y. K., Liao, P., Qiu, J. F., Lin, Y., Cai, X. F., Wang, D. Q., Hu, Y., Ren, J. H., Tang, N., Xu, Y. Y., Yu, L. H., Mo, Z., Gong, F., Zhang, X. L., Tian, W. G., Hu, L., Zhang, X. X., Xiang, J. L., Du, H. X., Liu, H. W., Lang, C. H., Luo, X. H., Wu, S. B., Cui, X. P., Zhou, Z., Zhu, M. M., Wang, J., Xue, C. J., Li, X. F., Wang, L., Li, Z. J., Wang, K., Niu, C. C., Yang, Q. J., Tang, X. J., Zhang, Y., Liu, X. M., Li, J. J., Zhang, D. C., Zhang, F., Liu, P., Yuan, J., Li, Q., Hu, J. L., Chen, J., and Huang, A. L. (2020). 'Antibody responses to SARS-CoV-2 in patients with COVID-19', Nat Med, 26: 845-848.

Molinaro, A. M. (2015). 'Diagnostic tests: how to estimate the positive predictive value', Neuro-Oncology Practice, 2: 162-166.

Özçürümez, M. K., Ambrosch, A., Frey, O., Haselmann, V., Holdenrieder, S., Kiehntopf, M., Neumaier, M., Walter, M., Wenzel, F., Wölfel, R., and Renz, H. (2020). 'SARS-CoV-2 antibody testing-questions to be asked', J Allergy Clin Immunol, 146: 35-43.

Pereira, P. (2016). 'Evaluation of rapid diagnostic test performance', Proof and Concepts in Rapid Diagnostic Tests and Technologies: 139-161.

Racine, R., and Winslow, G. M. (2009). 'IgM in microbial infections: taken for granted?', Immunol Lett, 125: 79-85.

Tang, M. S., Hock, K. G., Logsdon, N. M., Hayes, J. E., Gronowski, A. M., Anderson, N. W., and Farnsworth, C. W. (2020). 'Clinical Performance of Two SARS-CoV-2 Serologic Assays', Clin Chem, 66: 1055-1062.

Trevethan, R. (2017). 'Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice', Front Public Health, 5: 307.

Wang, J., Chen, C., Li, Q., Cai, P., Wang, Z., and Wang, L. (2020). 'COVID-19 confirmed patients with negative antibodies results', BMC Infectious Diseases, 20: 698.

Weissleder, R., Lee, H., Ko, J., and Pittet, M. J. (2020). 'COVID-19 diagnostics in context', Sci Transl Med, 12.

WHO. (2020a). "COVID-19 immune response." In.: World Health Organization.

———. (2020b). "Laboratory testing for coronavirus disease (COVID-19) in suspected human cases.

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., and Wendtner, C. (2020). 'Virological assessment of hospitalized patients with COVID-2019', Nature, 581: 465-469.

Yang, Y., Yang, M., Shen, C., Wang, F., Yuan, J., Li, J., Zhang, M., Wang, Z., Xing, L., and Wei, J. (2020). 'Laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections', MedRxiv.

Zhang, W., Du, R., Li, B., Zheng, X., Yang, X. L., Hu, B., Wang, Y., Xiao, G., Yan, B., and Shi, Z. "Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect. 2020; 9 (1): 386-9." In.

Zhao, J., Yuan, Q., Wang, H., Liu, W., Liao, X., Su, Y., Wang, X., Yuan, J., Li, T., Li, J., Qian, S., Hong, C., Wang, F., Liu, Y., Wang, Z., He, Q., Li, Z., He, B., Zhang, T., Fu, Y., Ge, S., Liu, L., Zhang, J., Xia, N., and Zhang, Z. (2020). 'Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019', Clin Infect Dis, 71: 2027-2034.

Niger. J. Physiol. Sci. 35 (December 2020): 123 - 129
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Plasma Atrial Natriuretic Peptide Responses to Salt-Loading in Salt-Sensitive and Salt-Resistant Normotensive and Hypertensive Nigerians

***Abidoye A.O.[1], Jaja S.I.[2] and Sofola O.A.[2]**

[1]*Department of Physiology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria*
[2]*Department of Physiology, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria*

Summary: Salt-sensitivity is more common in blacks than whites but the underlying cause is not fully known. Atrial natriuretic peptide (ANP) concentrations might play a role. This study investigated plasma ANP concentrations and effect of salt-loading in salt-sensitive (SS) and salt-resistant (SR) normotensive (NT) and hypertensive (HT) Nigerians of both genders. Forty-three (43) apparently healthy (NT) adult volunteers and thirty-seven (37) age-matched newly diagnosed (HT) Nigerians were grouped into SS and SR volunteers based on the mean changes in their mean arterial blood pressure ≥ 5 mmHg, following a 5-day administration of 200 mmol of sodium in each of the volunteers. ANP concentrations were determined before and after salt loading. Prevalence of SS and SR in the NT and HT Nigerians was 51.2% and 48.8%, respectively. Basal ANP levels in SS and SR NT and HT participants were similar but salt significantly raised ANP concentrations in SS ($p < 0.01$), SR ($p < 0.001$) NT volunteers only. Besides, basal ANP concentrations observed in SS and SR NT and HT males and females were similar but salt loading significantly increased ANP levels in SS NT males ($p < 0.05$), SR NT ($p < 0.001$) and HT ($p < 0.05$) females only. These findings showed that salt-sensitive hypertensive individuals demonstrated a blunted ANP response to salt loading. However, salt-resistant normotensive volunteers showed a significant increase in ANP concentrations, with higher levels in NT females than males. The impaired ANP response to salt challenge might be the basis for the higher prevalence of salt-sensitivity among blacks.

Keywords*: Salt-sensitivity, salt-resistance, atrial natriuretic peptide, gender and hypertension*

**Address for correspondence: abidoyedele@yahoo.co.uk; Tel: +234-8035020338*

Manuscript submitted: May 2020 Revised version accepted: October, 2020

INTRODUCTION

Hypertension is a chronic disease condition that is influenced by genetic and environmental factors (Kune and Zicha, 2009); and with the recent high consumption of dietary salt in most countries of the world, the prevalence of hypertension has increased tremendously (Choi *et al*., 2015; Dötsch-Klerk *et al*., 2015). Substantial evidence suggests that some individuals can adequately excrete high amounts of salt consumed without an appreciable increase in their arterial blood pressure whereas others cannot (Choi *et al*., 2015; Burnier, 2015). The underlying factor responsible for these differential blood pressure responses to salt loading and renal excretions of salt is not fully understood.

Salt-sensitivity can be simply defined as an increase in mean arterial blood pressure of 5 mmHg or above in response to increased salt intake (Felder *et al*., 2013; Elias *et al*., 2014). It increases with increased age, genetic predisposition, metabolic syndrome and obesity (Rust and Ekmekcioglu, 2017). Blacks are reported to be more salt-sensitive than whites (Burnier, 2008; Sandberg and Ji, 2012), About three-quarter (73%) of hypertensive African-Americans are reported to be salt- sensitive (Svetkey *et al*., 1996) while 25% of normotensive individuals are salt-sensitive (Franco and Oparil, 2006; Richardson *et al*., 2013). The prevalence of salt-sensitivity among adult Nigerians is high as it has been documented that more than 50 %of normotensive Nigerians are salt-sensitive and about 60% of hypertensive counterparts are salt-sensitive (Elias *et al*., 2011). Long-term implication of salt-sensitivity leads to increased mortality in both normotensive and hypertensive humans with no difference in their survival rate (Weinberger, 2002).

However, salt-sensitive hypertensive patients have 3-fold higher cardiovascular events than salt-resistant-hypertensive ones (Morimoto *et al*., 1997; Ehme, 2005). Besides, in males and females of comparable blood pressure values, the damaging effect of increased blood pressure on end organs is far greater in males than in females (Sandberg and Ji, 2012). Although, oestrogen has been documented to play a protective role on cardiovascular function in premenopausal women (Lorga *et al*., 2017), the basis for the greater adverse effect of sustained high blood pressure on the vital organs in males than females, is still a subject of intense research.

Atrial natriuretic peptide (ANP) is a cardiac endocrine hormone that regulates salt and water as well as blood pressure by promoting vasodilation, natriuresis and diuresis (Wang *et al*., 2012). It is secreted primarily from the cardiac atria in response to volume expansion. In mice, abnormally low ANP concentrations cause salt-sensitive hypertension (Song *et al*., 2015). The impaired renal excretory function that is implicated in salt-induced hypertension (Hall, 2016), might be due to abnormal concentrations of ANP in the susceptible individuals. There is paucity of data regarding

ANP concentrations in salt-sensitive normotensive and hypertensive Nigerians.

Hence, this study was designed to investigate plasma ANP concentrations in salt-sensitive and salt-resistant normotensive and hypertensive adult Nigerians, as well as to determine effect of salt and gender on ANP levels in these individuals.

MATERIALS AND METHODS

The study was conducted on forty- three (43) apparently healthy normotensive and thirty- seven (37) age-matched hypertensive volunteers. The number of participants was statistically determined according to the formula prescribed by Eng *et al.,* 2003 for a comparative study. The volunteers were briefed about the experiment and informed consent was obtained. Ethical approval to carry-out the study was obtained from the Health Research Ethic Committee of the College of Medicine, University of Lagos. The participants were divided into salt-sensitive and resistant normotensive and hypertensive groups based on the mean changes in their mean arterial blood pressure (MAP) of 5mmHg or above following 5-day period of salt administration (Weinberger, 1996; Elias *et al.,* 2014).

Inclusion Criteria: The normotensive volunteers that were included in the study had BP < 140/90 mmHg. They were not diabetic or smokers of cigarettes or suffering from any cardiovascular, cerebrovascular or renal disease. The hypertensive volunteers had sustained systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or both (Franklin, 2004). They were not on any antihypertensive drug and not suffering from any complication arising from their sustained elevated blood pressure.

Exclusion Criteria: Volunteers who had severe high blood pressure (BP ≥ 180/110 mmHg), abnormal ECG findings or abnormally high plasma potassium (K ≥ 5.5 mmol) or creatinine levels (Cr ≥ 110μmol/l) were excluded from participating in the study. Pregnant women were also excluded from the study due to medical and ethical reasons. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP), plasma atrial natriuretic peptide (ANP), plasma and urinary concentrations of sodium, potassium, urine volumes as well as 24- hour urinary excretions of sodium were determined before and after salt loading in the participants.

Determination of Blood Pressure: Resting blood pressure was determined by auscultatory method, using Accoson Mercury Sphygmomanometer (Accoson, United Kingdom) as per the described instructions of American Heart Association (Beevers *et al*., 2001). Volunteers were allowed to rest for 10 minutes in sitting position before the commencement of measurements. Appropriate cuff sizes were applied based on their mid-arm circumferences. This was ensured so as to prevent under-cuffing or over- cuffing, as this could adversely affect the blood pressure readings. The cuff was wrapped on the right arm of each of the subjects with the midline of bladder over the brachial arterial pulsation.

The cuff bladder was inflated rapidly while palpating radial pulse. Reading at which pulse disappeared was noted, pressure was further elevated by 20-30 mmHg above this value. Then the bladder was slowly deflated while listening for the appearance of Korotkoff's sounds using a stethoscope, placed on the brachial arterial pulsation. Systolic blood pressure and diastolic blood pressure were recorded to the nearest 2mmHg, as the first appearance and disappearance of Korotkoff's sounds, respectively. The blood pressure was measured thrice in each of the participants and average of the readings was determined and recorded.

The mean arterial blood pressure (MABP) was determined from the sum of diastolic blood pressure and one-third of pulse pressure (Zheng *et al*; 2008). The pulse pressure is the difference between systolic and diastolic blood pressure.

Protocol for venous blood collection: Subjects fasted overnight and reported at 9 am in the laboratory for the collection of their venous blood. They were briefed about the procedure. Venous blood was withdrawn from one of the antecubital veins under aseptic condition and emptied into appropriately labeled blood sample bottles. Lithium heparin bottles were used for plasma sodium estimation while chilled EDTA bottles were used for ANP analysis

The withdrawn blood samples were spun immediately at 3,000 rpm at 4ºC for 10 minutes. The supernatants were stored at -40ºC until analyses were carried-out.

Collection of 24-Hour Urine Specimen: The participants were briefed about the procedure and given a 5 litre- plastic keg each to collect their 24-hour voided urine after they had emptied their bladders in the morning. The urine was brought to the laboratory the following day in the morning and measured with a measuring cylinder in order to determine the volumes. Aliquots of the urine were put in the universal bottles and stored at -20^0C until analyses. On the day of analyses, the frozen urinary samples were allowed to thaw at room temperature.

The 24- hour urinary concentrations of sodium and potassium were measured using ion selective electrode (ISE 6000) machine (SFRI, France). The 24- hour urinary excretions of Sodium and potassium were determined by multiplying the concentrations of urinary sodium and potassium in mmol/day by the urinary volumes in litres/day, respectively (Land *et al.,* 2014).

Salt loading in the study Participants: Having determined and recorded the baseline blood pressure and laboratory parameters in the study participants, they ingested a salt load of 200 mmol of sodium each per day in two divided doses for 5 days (Tzemos *et al*; 2008; Elias *et al;* 2014). Compliance of the volunteers with salt ingestion was assessed by determining their 24-hour urinary excretion of sodium before and after salt loading.

Determination of Laboratory Parameters Measured in the study population: The laboratory parameters that were determined in the normotensive and hypertensive subjects were plasma atrial natriuretic peptide (ANP), plasma and urinary concentrations of sodium, potassium as well as 24-hour urinary volumes and excretions of sodium and

potassium. They were determined before and after salt loading in the participants.

Plasma ANP concentrations were determined using Human Atrial Natriuretic Peptide Eliza Kits (Sunlong Biotech, China). The ANP concentrations were analyzed as described by the manufacturer's instructions.

Plasma sodium and potassium concentrations were determined using ion selective electrode (ISE 6000) machine (SFRI, France). The 24- hour urinary volumes and excretions of sodium and potassium were also measured.

Determination of Salt-Sensitivity and Salt-Resistance in the Study Population: The normotensive and the hypertensive volunteers that showed changes of 5 mmHg or greater in their mean arterial blood pressure after salt loading were considered salt-sensitive participants while those that demonstrated less than 5 mmHg were designated, salt- resistant participants (Weinberger, 1996; Elias *et al.*, 2014).

Data Analysis

Data analysis was carried-out with the aid of GraphPad Statistical software, Version 5 for Windows (GraphPad Software, San Diego, California, USA). Data was expressed as mean ± standard error of the mean. The prevalence of salt-sensitivity and resistance in the study groups was expressed in percentages. Variations within or between the salt-sensitive and salt-resistant normotensive and hypertensive volunteers were analyzed using appropriate Student's t- test. Statistical significance was accepted at $p < 0.05$ level.

RESULTS

Effect of Salt Loading on Urinary and Plasma Electrolytes in the Study Population: Table 1 shows urine volumes, plasma and urinary concentrations of sodium and potassium as well as urinary excretions of the electrolytes before and after salt loading in the normotensive and hypertensive volunteers.

The basal values of the urinary and plasma sodium and potassium concentrations observed in the normotensive and hypertensive participants were similar before salt loading. However, after salt loading, urinary sodium concentrations and urinary excretions of sodium in both normotensive and hypertensive volunteers were significantly increased ($p < 0.001$) but plasma sodium concentrations were not elevated in the normotensive subjects but increased ($p < 0.05$) in the hypertensive counterparts.

Besides, plasma concentrations of sodium in the hypertensive participants were significantly higher ($p < 0.05$) than their normotensive volunteers after salt loading. In addition, plasma potassium concentrations were decreased ($p < 0.05$) by salt in the hypertensive volunteers but not significantly in the normotensive counterparts. Furthermore, after acute salt loading, the plasma potassium levels observed in the hypertensive subjects were significantly less ($p < 0.05$) than those of the normotensive colleagues. Besides, urine volumes (output) were increased ($p< 0.05$) by salt challenge in the normotensive volunteers but not significantly in their hypertensive counterparts (Table 1).

Prevalence of Salt-Sensitivity and Salt-Resistance in the Study Population: Table 2 shows the prevalence of salt sensitivity and salt resistance in the normotensive and hypertensive participants. More than half (51.2%) of the normotensive volunteers were salt-sensitive and salt-sensitivity was commoner in males than females (27.9% vs23.3%). The rest (48.8%) of the normotensive volunteers were salt-resistant. In addition, in the normotensive group, females were more salt-resistant than males (30.2% vs 18.6%). In hypertensive group, nearly half (48.6%) of the participants were salt-sensitive. However, salt sensitivity was observed to be more common in females than males (27.0% vs21.6%). The rest (51.4%) of the hypertensive volunteers were salt-resistant. More females than males were found to be salt-resistant (29.7% vs 21.6%, Table 2). Table 2 shows that combination therapy (HU + L-Arg) caused an elevation of antioxidant enzymes levels ($p < 0.001$ in each case) but lowered MDA ($p < 0.001$) and each of the liver enzymes levels ($p < 0.001$ in each case).

Table 1:

Urinary Output, Concentrations and Excretions of Sodium and Potassium and Plasma Levels of Sodium and Potassium before and after Salt Loading in the Study Population

Laboratory Parameters	Normotensive group (n =43)		Hypertensive Group (n = 37)		p Value			
	Before Salt (a)	After Salt (b)	Before Salt (c)	After Salt (d)				
					(a) Vs (b)	(a) vs (c)	(c) vs (d)	(b) vs (d)
PNa (mmol/l)	134.9±0.46	135.3± 0.06	135.7± 0.55	136.7±0.31	NS	NS	< 0.05	< 0.05
PK (mmol/l)	4.56± 0.06	4.53±0.07	4.46± 0.66	4.31± 0.06	NS	NS	< 0.05	< 0.05
24-Hr UV (l)	1.77± 0.11	2.03± 0.12	1.59± 0.13	1.87± 0.11	< 0.05	NS	NS	NS
UNa (mmol/l)	93.15±5.91	132.00±8.33	91.49±6.82	137.3±7.23	< 0.001	NS	< 0.001	NS
UK (mmol/l)	23.31± 2.09	19.54± 1.95	23.85± 2.13	23.30±2.36	NS	NS	NS	NS
UENa (mmol/day)	155.0± 9.56	243.4±14.01	130.8±10.55	246.5±15.63	< 0.001	NS	< 0.001	NS
UEK (mmol/day)	37.49±3.03	34.66±2.63	32.44± 2.77	39.44± 2.89	NS	NS	NS	NS

Values are expressed as Mean ± SEM as analyzed by paired t- test

Key:

PNa = plasma sodium; PK = plasma potassium; UNa= urinary sodium; UK = urinary potassium; 24-Hr UV =24-hour urine volume: UENa = urinary excretion of sodium; UEK = urinary excretion of potassium; before salt = before salt loading; after salt = after salt loading; p< 0.05, p <0.01 and p < 0.001 = significant; NS =not significant

Table 2:
Prevalence of Salt-Sensitivity in the Study Population

	Normotensive Participants		Hypertensive Participants	
Gender	Salt-Sensitive	Salt-Resistant	Salt-Sensitive	Salt-Resistant
Males n, %	12 (27.9%)	8 (18.6%)	8 (21.6%)	8 (21.6%)
Females (n, %)	10 (23.3%)	13 (30.2%)	10 (27.0%)	11 (29.7%)
Total	22 (51.2%)	21 (48.8%)	18 (48.6%)	19 (51.4%)

Values are expressed as numbers and percentages
n = number of participants

ANP Concentrations in Salt-Sensitive and Salt-Resistant Normotensive and Hypertensive Participants: The plasma concentrations of ANP in salt-sensitive and salt-resistant normotensive and hypertensive volunteers are shown in Figure 1. Basal ANP concentrations in salt-sensitive and salt-resistant normotensive and hypertensive participants were similar before salt loading. However, salt significantly elevated ANP levels in salt-sensitive ($p < 0.01$) and salt-resistant ($p < 0.001$) normotensive volunteers.

Besides, the mean change (Δ) in ANP concentrations in salt-resistant normotensive participants was significantly higher ($p < 0.01$) when compared with that of the salt-sensitive normotensive colleagues. (Figure 1). In the hypertensive group of volunteers on the other hand, the basal ANP levels observed in the salt-sensitive and salt-resistant participants, were not significantly different and salt did not significantly raise their ANP levels. Besides the mean changes in their ANP concentrations were similar (Figure 1).

Gender Influence on ANP Concentrations in Salt-Sensitive and Salt- Resistant Normotensive and Hypertensive Participants: Figure 2 shows ANP levels before and after loading in salt-sensitive and salt-resistant male and female normotensive participants. Their basal plasma ANP concentrations were seen to be similar before salt loading, However, salt loading significantly raised ANP levels ($p < 0.05$) in both salt-sensitive and salt-resistant normotensive males. Besides, there was also a significant rise ($p < 0.001$) in ANP concentrations observed in salt-resistant normotensive female volunteers after salt loading but. no significant increase was observed in salt-sensitive normotensive female participants (Figure 2). In the hypertensive subjects on the other hand, the basal ANP levels in salt-sensitive and salt-resistant males and females were not significantly different. However, after salt loading, the ANP levels were significantly raised ($p < 0.05$) in the salt-sensitive hypertensive males and salt- resistance hypertensive females but not significantly in salt-sensitive hypertensive females and salt-resistant hypertensive males (Figure 3).

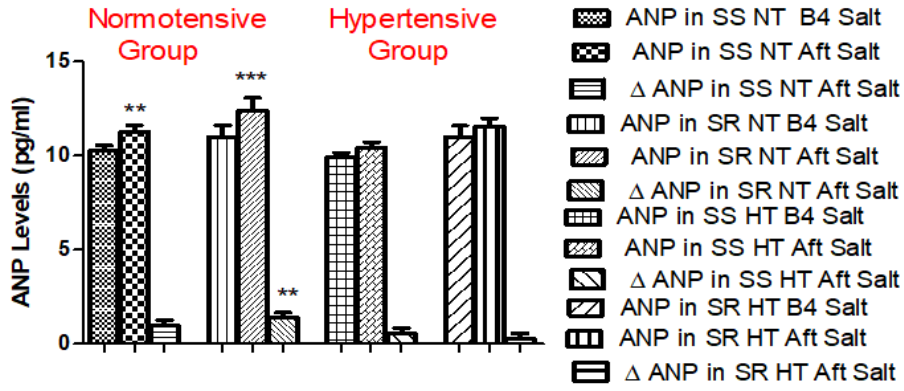


Figure 1
The plasma ANP concentrations in salt-sensitive and salt-resistant normotensive and hypertensive participants before and after salt loading.
NT = normotensive; HT = hypertensive; SS = salt-sensitive; SR = salt-resistant;
Δ = change
B4 salt = before salt loading; Aft salt = after salt loading
** = p< 0.01 between ANP concentration before and after salt loading in SS normotensive participants.
***= p < 0.001between ANP concentrations before and after salt loading in SR normotensive participants.
** = p < 0.01 between the mean changes (Δ) in ANP concentrations after salt loading in SS and SR normotensive participants

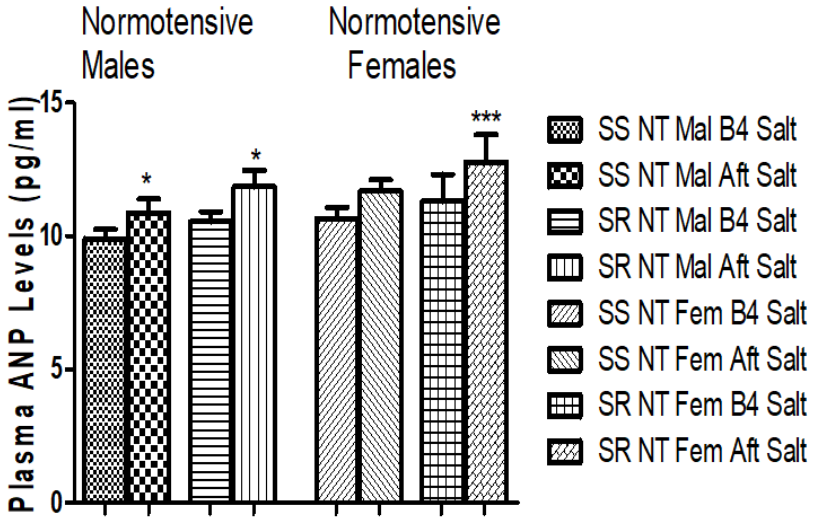


Figure 2
The plasma ANP levels before and after salt loading in salt-sensitive and salt-resistant normotensive male and female participants.
NT = normotensive; SS = salt-sensitive; SR = salt-resistant; Mal = males; Fem = females
B4 salt = before salt loading; Aft salt = after salt loading;
* = p < 0.05 between ANP levels before and after salt loading in salt-sensitive normotensive male participants
* = p < 0.05 between ANP levels before and after salt loading in salt-sensitive normotensive male participants
*** = p < 0.001between ANP levels before and after salt loading in salt-resistant normotensive female participants

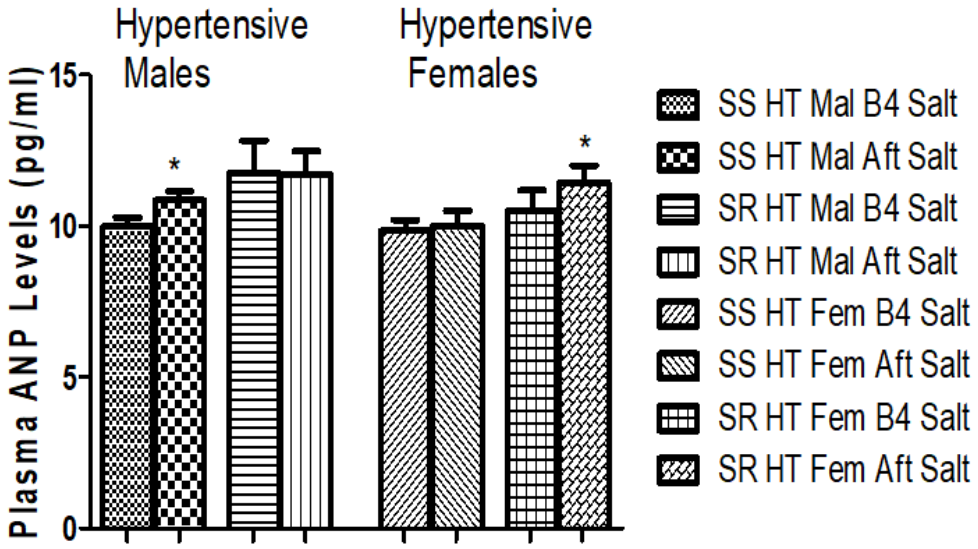


Figure 3
The plasma ANP levels before and after salt loading in salt-sensitive and salt-resistant hypertensive male and female participants
*HT = hypertensive; SS = salt-sensitive; SR = salt-resistant; Δ = change; * = p < 0.05*
B4 salt = before salt loading; Aft salt = after salt loading; Mal = males; Fem = females
** = p < 0.05 between ANP levels before and after salt loading in salt-sensitive hypertensive male participants*
** = p < 0.05 between ANP levels before and after salt loading in salt-resistant hypertensive female participants*

DISCUSSION

The selection of the volunteers was primarily based on their health status. The hypertensive volunteers were newly diagnosed. They did not suffer from any complication arising from their hypertensive state as abnormally high ANP levels have been documented to be seen in heart and kidney diseases (Ogawa et.al, 2015; Volpe *et al*., 2016). In addition, compliance with the salt ingestion by the volunteers was good as evident by the significant increases observed in the 24-hour urinary excretions of sodium in the study population.

Hypertensive volunteers had higher basal plasma sodium levels than their normotensive counterparts. The plasma sodium concentrations in the study population were significantly elevated after salt loading. In addition, the hypertensive subjects had appreciably lower plasma potassium levels than normotensive colleagues following salt loading. High plasma sodium and low plasma potassium concentrations have been implicated in the pathogenesis of hypertension (Abcar and Kujubu, 2009; Perez and Chang, 2014). The increased plasma sodium concentrations and decreased urinary loss of water possibly promoted increases in their blood volumes and cardiac output thereby elevating their arterial blood pressure.

The prevalence of salt-sensitivity as seen in this study population was high, indicating the vulnerability of these salt-sensitive normotensive individuals to the development of salt-sensitive hypertension and cardiovascular disease (Wang *et al*., 2012). Although, the prevalence of salt-sensitivity has been previously documented to be lower in normotensive individuals than hypertensive ones (Franco and Oparil, 2006; Elias *et al*., 2014), the percentage of normotensive volunteers that was observed to be salt-sensitive in this study was slightly higher than that of their hypertensive counterparts. Although, the difference in the prevalence might be due to sample size, it may also stem from increased consumption of salt from various dietary sources which have now become a norm in most countries (Dötsch-Klerk *et al*.,2015).

Nevertheless, in a normotensive state, salt-sensitivity was commoner in males than females whereas the reverse was the case under a hypertensive condition as salt-sensitivity was found to be commoner in hypertensive females than males. The lower prevalence of salt-sensitivity in the normotensive females than males might be due to a protective role that their oestrogen plays on their cardiovascular function (Lorga *et al*., 2017). This protective effect of oestrogen seems to be impaired or attenuated in a hypertensive state. This could be responsible for the higher prevalence of salt-sensitivity that was observed in the hypertensive females.

The basal ANP concentrations in salt-sensitive and salt resistant normotensive and hypertensive adult Nigerian subjects who had not developed any complication from their hypertensive condition, were similar. However, when confronted with salt loading, ANP appreciably rose in the normotensive participants with a greater response in salt-resistant normotensive volunteers but salt-sensitive normotensive and hypertensive volunteers did not show any significant rise.in their ANP levels. The slight increase in ANP concentrations observed in these individuals might be the basis for their salt-sensitivity. Abnormally low ANP has been implicated in pathogenesis of salt-sensitive hypertension in mice (Wang *et al*., 2012; Song *et al*., 2015). Regarding the influence of gender on the plasma ANP concentrations in the study salt-sensitive and salt-resistant normotensive and hypertensive volunteers, salt- resistant normotensive female participants demonstrated greater plasma ANP response to salt loading when compared with their counterpartsalt-sensitive normotensive or hypertensive males. The ANP levels were also found to be higher than that of their salt-resistant hypertensive female colleagues. The gender difference in their ANP concentrations as seen in the normotensive females might also be due to oestrogen effect on the ANP concentrations as it has been reported that

oestrogen induces ANP release from the heart via oestrogen receptor (Vishwakarma *et al*., 2016). However, it is not very clear why salt-sensitive normotensive females demonstrated no significant increase in their ANP levels after salt loading. The slight increase in the plasma ANP levels following salt loading in these normotensive individuals might likely be the cause of their salt-sensitivity.

Though, it is reported and it was also observed in this study that hypertensive participants demonstrated a blunted ANP response to salt loading (Campese *et al*., 1996), this finding was not the case in all the hypertensive volunteers who were salt loaded in this study. Salt-resistant hypertensive females still showed a significant increase in their plasma ANP concentrations after the salt challenge. This implies that the aetiology of hypertension in these hypertensive individuals might not be primarily due to inadequate salt-handing arising from abnormally low ANP levels. The pathogenesis of hypertension in these individuals might be multifactorial.

In conclusion, the study has shown that basal plasma atrial natriuretic peptide levels in salt-sensitive normotensive and hypertensive Nigerians who had not developed any complication were similar but the concentrations of the hormone were significantly raised by salt loading in salt-sensitive and salt-resistant normotensive volunteers with a greater increase in the later. Salt-sensitive hypertensive participants demonstrated blunted ANP response to acute salt-loading. This finding might be the basis for the higher prevalence of salt-sensitivity documented to be seen among blacks

Acknowledgment

The Tertiary Education Trust fund (TETfund) Nigeria is appreciated for its financial support.

REFERENCES

Abscar, A.C. and Kujubu, D.A. (2009). Evaluation of hypertension with hypokaelemia. Perm J. 13(1): 73 -76.

Beevers, G., Gregory, Y.H., and O'Brein, E. (2001). Blood pressure measurement: Part II conventional sphygnomanometry: technique of auscultatory blood pressure measurement. BMJ; 322: 1043-1047.

Burnier, M. (2008). Ethnic differences in renal handling of water and solutes in hypertension. Hypertension,52:203 – 204.

Burnier, M., Wuerzner, G. and Bochud, M. (2015). Salt blood pressure and cardiovascular risk: What is the most adequate preventive strategy? A Swiss perspective. Front Physiol. 6: 227

Campese, V.M., Tawadrous, M.T., Bigazzi, R., Bianchi, S., Mann, A.S., Oparil, S. and Raij. L. (1996). Salt intake and plasma atrial natriuretic peptide and nitric oxide in hypertension. Hypertension, 28(3): 3335 – 3345.

Choi, H.Y., Park, H.C. and Ha, SK. (2015). Salt-sensitivity and hypertension: A paradigm shift from kidney malfunction to vascular endothelial dysfunction. Electrolyte Blood Pressure, 13(1): 7 – 16.

Dötsch-Klerk, M., Goossens, W.P., Meijer, G.W. and van het Hof, K.H. (2015). Reducing salt in food; setting product-specific criteria aiming at a salt of 5 g per day. European Journal of Clinical Nutrition, 69(7): 799 – 804.

Ehmke, H. (2005). Neurogenic mechanisms and salt sensitivity. Hypertension, 46: 1259 – 1260.

Elias, S.O., Azinge, E.C., Umoren, G.A., Jaja, S.I. and Sofola, O.A. (2011). Salt-Sensitivity in Normotensive and Hypertensive Nigerians. Nig Q J Hosp Med; 21(1): 85 – 91.

Elias, S.O., Sofola, O.A. and Jaja, S.I. (2014). Vascular reactivity and salt sensitivity in normotensive and hypertensive Nigerians. J. Afr. Ass. Physiol. Sci; 2 (2): 95-103.

Felder, R.A., Marquitta, J., Scott, M.W., Pedro, A.J. (2013). Diagnostic tools for hypertension and salt sensitivity testing. Curr Opin Nephro Hypertens 22(1):65 – 76.

Franklin, S.S. (2004). Systolic blood pressure: it is time to take control. American Journal of hypertension; 17 (S3): 49S -54S.

Franco, V. and Oparil, S. (2006). Salt-sensitivity, a determinant of blood pressure, cardiovascular disease and survival. J Am Coll Nutr; 25(3): 247S – 255S.

Hall, J.E. (2016). Kidney dysfunction mediates salt induced increases in blood pressure. Circulation, 133(9).894 – 906.

KuneJ, and Zicha, J. (2009). The interaction of genetic and environmental factors in the etiology of hypertension. Physiol Res 58 (2): S33 – 41.

Land, M-A., Webster, J., Christoforou, A., Praveen, D., Jeffery, P., Chalmers, J., Smith, W., Woodward, M., Barzi, F., Nowson, C., Flood, V. and Neal, B. (2014). Salt intake assessed by 24 h urinary sodium excretion in a random and opportunistic sample in Australia. BMJ Open;4: e003720.

Lorga, A., Cunningham, C.M., Moazeni, S., Ruffenach, G., Umar, S., Eghbali, M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. Biol Sex Differ; 8: 33.

Morimoto, A., Uzu, T., Fujii, T., Nishimura, M., Kuroda, S., Nakamura, S., Inenaga, T., Kimura, G., (1997). Sodium sensitivity and cardiovascular eventsin patients with essential hypertension. Lancet, 350: 734 – 1737.

Ogawa, N., Komura, H., Kuwasako, K., Kitamura, K. and Kato, J. (2015). Plasma levels of natriuretic peptides and development of chronic kidney disease. BMC Nephrology; 16:171.

Perez, V. and Chang, E. (2014). Sodium to potassium ratio and blood pressure, hypertension and related factors. Adv Nutr 5(6): 712 – 741.

Richardson, S.I., Freeman, B.I., Ellison, DH., Rodriguez, C.J. (2013). Salt sensitivity: a review with a focus on non-Hispanic blacks and Hispanics. J Am Soc of Hypertens; 7(2): 170–179.

Rust, P. and Ekmekcioglu, C. (2017). Impact of salt intake on the pathogenesis and treatment of hypertension. Adv Exp Med Biol; 956: 61 – 84.

Sandberg K and Ji H. (2012). Sex differences in primary hypertension. Biol Sex Differ; 3(1):7/.

Song, W., Sang, H. and WU, Q. (2015). Atria natriuretic peptide in cardiovascular biology and disease (NPPA). Gene; 569 (1): 1 – 6.

Strazzulo, P., Barbato, A., Vuotto, P. and Galletti, F. (2001). Relationship between salt sensitivity of blood pressure and sympathetic nervous system activity: A short review

of evidence. Clinical and Experimental Hypertension 23(1-2): 25- 33.

Svetky, L.P., Mckeown, S.P. and Wilson, A.F. (1996). Heritability of salt sensitivity in black Americans. Hypertension, 28(5): 854 – 8.

Tzemos, N., Lim, P.O., Wong, S., Struthers, A.D. and MacDonard, T.M. (2008). Adverse cardiovascular effects on acute salt loading in young normotensive individuals. Hypertension; 51(6):1525 – 1530.

Vishwakarma, V.K., Gupta, J.K., Qureshi, S.S. and Agrawal, V. (2016). Role of atrial natriuretic peptide in various conditions. Int J Pharma Bio Sci; 7 (3): 20 – 27.

Volpe, M., Carnovail, M. and Mastromarino, V. (2016). The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis of treatment. Clin Sci 2016; 130 (2):57 – 77.

Wang, W., Shen, J., Cui, Y., Jiang. J. *et al*(2012). Impaired sodium excretion and salt-sensitive hypertension in corin-deficient mice. Kidney Inter;82(1):26 – 33.

Weinberger, M. (1996). Salt-sensitivity of blood pressure in humans. Hypertension ;27: 481-490.

Weinberger, M.H. (2002). Salt sensitivity is associated with an increased mortality in both normal and hypertensive humans. J Clin Hypertens, 4(4): 274 – 6.

Zheng, L., Sun, Z., Li, J., Zhang, R., Zhang, X. *et al*. (2008).Pulse pressure and mean arterial pressure in relation to ischaemic stroke among patients with uncontrolled hypertension in rural areas of China. Stroke; 39(7): 1932 – 1937.

Niger. J. Physiol. Sci. 35 (December 2020):131 - 134
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Blood Pressure, Haematologic and Biochemical Changes Following L-Arginine Supplementation in Children with Sickle Cell Anaemia already on Hydroxyurea Therapy

Temiye E.O.[1], Eimunjeze O.P.[2], Ogungbemi S.I.[2] and Jaja S.I.[2*]

Departments. of [1]Pediatrics and [2]Physiology, College of Medicine, University of Lagos. Lagos, Nigeria

Summary: Hydroxyurea is an approved therapy for management of children suffering from sickle cell disease (SCD). In adult sufferers, co-administration of hydroxyurea and l-arginine had shown some benefits. This study examined the effect of co-administration of hydroxyurea (15-35mg/kg/day) and l-arginine (500 mg/day) for 6 weeks on blood pressure and haematological indices, liver and antioxidant enzymes levels. The levels of these parameters when the subjects were on hydroxyurea alone were taken as control values. Results showed that combined therapy (HU + l-arginine) decreased SBP, DBP, MAP and PP ($p < 0.01$ respectively) but enhanced %HbF, Hb and PCV ($p < 0.001$ in each case). It elevated CAT, SOD, GPX ($p < 0.001$ in each case) but depressed MDA, AST, ALT and ALP ($p < 0.001$ respectively). Study thus showed that combined therapy (HU + l-arginine) provided more useful benefits to children suffering from sickle cell anaemia than hydroxyurea alone.

Keywords*: Hydroxyurea, L-Arginine, children, sickle cell anaemia*

**Address for correspondence*: sjaja@unilag.edu.ng; *Tel: +234-803 672 9192*

Manuscript received: August, 2020.
Revised version accepted: November 2020.

INTRODUCTION

Sickle cell disease (SCD) results from a single mutation in the beta globin chain inducing the substitution of valine for glutamic acid at the sixth amino acid position. This mutation leads to the production of abnormal haemoglobin S (HbS) (Pauling *et al*, 1949; Ingram, 1956). The sickle cell gene is dispersed around the world and consequently is recognized as a world-wide problem due to its impact on mortality and morbidity. The incidence of this molecular disease in Africa is between 5 – 40%. In Nigeria it is estimated that there are about 88,000 new births annually and about 160,000 new births per annum in Africa. Apart from sub-Saharan Africa where the disease is most frequently found, it is also found in the United States of America, Brazil, Greece Turkey (Diallo and Tchernia, 2002; Piel *et al*, 2013).

Polymerization of the abnormal haemoglobin (HbS) has clinical manifestations. These include chronic haemolytic anaemia resulting in sickle cell anaemia (SCA) and periodic microcirculatory vaso-occlusion which gives rise to high oxidative stress burden, tissue ischemia and pain. Eventually, there is chronic end-organ damage reducing the lifespan of the individual (Hebbel *et al*, 2009).

Hydroxyurea (hydroxycarbamide) and Endari (L-glutamate) are approved therapies for SCD in adults and children (Carden and Little, 2019). Hydroxyurea induces hemoglobin F (HbF) and increases red blood cell volume, thus reducing the chances of polymerization of HbS. In addition, it elevates the level of nitric oxide in plasma thus decreasing mortality and morbidity (McGann and Ware, 2015).

Arginine participates in many biochemical activities of normal physiology. It is a basic amino acid that occurs naturally and found in proteinous foods like meat, poultry, nuts and fish and also in watermelon. L-Arginine supplementation (1g/day for 6wks) in adults normalized the blunted hemodynamic changes associated with posture adjustments in HbSS subjects. The effect of arginine was attributed to increased nitric oxide metabolites (NO_X) (Ogungbemi *et al*, 2013). L-Arginine had also been shown in SCA sufferers to lower elevated liver enzymes and malondialdehyde, but elevating nitric oxide metabolites (NO_X) (Jaja *et al*, 2016).

Some studies had suggested that l-arginine could enhance the therapeutic effects of hydroxyurea when they are co-administered. Morris *et al*. (2003) showed that arginine when given acutely did not increase serum NOx production in SCD patients at steady state, but it did when given together with hydroxyurea. In addition, Eleuterio *et al*, (2019) had shown that co-administering hydroxyurea and l-arginine for four months increased serum NOx levels and relieved pain, thus demonstrating that co-administration of the two drugs may be useful for pain relief and thus improving the quality of life of these patients.

Sickle cell disease eventually affects all systems of the body. However, the effect of a combination of hydroxyurea and l-arginine on several systems of the body has not been investigated in children suffering from sickle cell anaemia. This study investigated the effect of a combination of hydroxyurea and l-arginine on blood pressure, haematologic indices, liver and antioxidant enzymes on children suffering from sickle cell anaemia that were already on hydroxyurea therapy.

MATERIALS AND METHODS

Ethical consideration: Institutional approval was obtained from The Research Grants and Experimentation Ethics Committee of the College of Medicine, University of Lagos, Lagos, Nigeria (CM/COM/8/VOL.XXIII). A parent of each child that participated in the study gave informed consent before the start of the study.

Study population and location: Thirty seven (37) children were recruited for the study. However twenty two (22) children completed the study. Fifteen (15) children were unable to complete the study especially due to school examinations. The children were of both sexes aged between 3 years and 12 years. They were previously confirmed to be suffering from sickle cell anaemia and thus regularly attending the Pediatrics Outpatient Clinic of the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria.

Data collection: Each participating patient was already on hydroxyurea therapy (15 -35 mg/kg/day). Age (yrs) was recorded. Height, (m) and weight (kg) were measured and recorded. Blood pressure was measured before withdrawal of five milliliter of blood from an anti-cubital vein of the patient. Part of the collected blood was stored in anticoagulant bottles for the measurement of haematological indices while the other part was centrifuged, serum collected in plane bottles for the estimation of liver enzymes, antioxidant enzymes and malondialdehyde (lipid peroxidation). Each patient then received 500 mg/day of l-arginine (Mason Vitamins Inc. Miami Lakes, Florida, USA) for six (6) weeks. L-Arginine served as an addition to hydroxyurea therapy. Re-evaluation of the parameters followed after six weeks of combined therapy.

Blood pressure measurement: Auscultatory method was employed in the measurement after the subject had taken a 15-minute rest. Arterial blood pressure was measured on the brachial artery of each subject in the supine position. Disappearance of Korotkoff's sound (fifth phase) marked diastole. Pulse pressure was the difference between systolic blood pressure and diastolic blood pressure. Mean arterial blood pressure was calculated as diastolic blood pressure plus one third of pulse pressure.

Estimation of hematological parameters: Percent foetal haemoglobin (%HbF) and packed cell volume (PCV) were estimated with the microhaematocrit method. Haemoglobin concentration ([Hb] (g/dl) was determined with the colorimetric method (Dacie and Lewis, 1991).

Measurement of liver enzymes: Serum liver enzymes (aspartate amintransferase, AST, IU/L), alanine aminotransferase (ALT, IU/L) and alkaline aminotransferase (ALP, IU/L) were measured with colorimetric method utilizing RANDOX® kits.

Estimation of malondialdehyde: Malondialdehyde was measured with the spectrophotometer using the thiobarbituric acid method (Titus *et al*, 2004).

Determination of blood levels of antioxidant enzymes: Serum CAT and SOD levels were assayed as described by Rukkumani *et al*, (2004) while serum GPx level was measured as described by Ellman (1959).

Data analyses

Results have been expressed as mean ± standard error of mean ($\chi \pm$ SEM). Paired t' test was used in making statistical comparisons. Significance was accepted when $p < 0.05$.

RESULTS

Children were between the ages of three (3) years and twelve (12) years (Mean ± SEM = 5.60 ± 1.1 years). Their heights ranged from 1.0 m to 1.42 m (1.2 ± 0.05 m). Mean weight slightly increased from 18.6 ± 2.6 kg (range 10.0 kg – 35.0 kg) at the beginning of the study to 19.7 ± 2.2 kg (range 10.0 kg – 38.0 kg) at the end of the study. Mean body mass index (BMI) also increased slightly from 16.4 ± 0.6 kg/m^2 (range 14.0 kg/m^2 to 17.4 kg/m^2) to 17.2 ± 0.4 kg/m^2 (range 13.8 kg/m^2 to 18.0 kg/m^2) within the 6-week study period.

Table 1 compares blood pressure responses to hydroxyurea (HU) therapy alone with the combination (HU + L-Arg) therapy. Combination therapy significantly reduced the blood pressure parameters ($p < 0.001$ in each case) except pulse pressure. Table 1 also shows that combination therapy significantly elevated each of the hematological parameters ($p < 0.001$ in each case).

Table 1:
Comparison of blood pressure and haematological parameters before and after L-Arginine supplementation.

Parameters	HU alone Mean ± SEM (Range)	HU + L-Arg Mean ± SEM (Range)	Δ (mm Hg)	Δ (%)	P level
SBP (mm Hg)	101.1 ± 5.7 (60.0 – 123.0)	89.8 ± 4.5 (58.0 – 120.0)	- 11.2 ± 1.8	- 11.2 ± 1.6	< 0.01
DBP (mm Hg)	65.9 ± 3.5 (40.0 – 98.0)	56.9 ± 2.8 (40.0 - 70.0)	- 8.9 ± 2.6	-13.6 ± 3.2	< 0.01
MAP (mm Hg)	77.6 ± 4.0 (46.6 – 106.3)	67.8 ± 3.2 (50.6 – 87.8)	- 9.8 ± 2.2	-12.6 ± 2.4	< 0.01
PP (mm Hg)	35.2 ± 3.0 (15.0 – 40.0)	32.1 ± 1.9 (15.0 – 40.0)	- 3.0 ± 2.2	-1.0 ± 7.0	NS
HbF (%)	5.3 ± 0.5 (1.6 – 11.9)	7.0 ± 0.5 (3.7 – 13.2)	1.7 ± 0.4	32.1 ± 5.6	< 0.001
Hb (g/dl)	7.9 ± 0.3 (4.8 – 10.9)	9.6 ± 0.2 (6.1 – 12.3)	1.7 ± 0.2	21.6 ± 4.8	< 0.001
PCV (%)	22.4 ± 0.7 (13.7 – 27.3)	27.6 ± 0.7 (17.7 – 32.5)	5.3 ± 0.3	23.5 ± 2.8	< 0.001

KEY

SBP = Systolic blood pressure; DBP = Diastolic blood pressure; PCV = Packed cell volume ; HU = Hydroxyurea MAP = Mean arterial pressure; L-Arg = L-Arginine; PP = Pulse pressure ; Δ = Change; HbF = Hemoglobin F; %Δ = Percent change; Hb = Hemoglobin; NS = Not Significant

Table 2:
Effect of combination therapy on malondialdehyde, antioxidant and liver enzymes.

Parameters	HU alone Mean ± SEM (Range)	HU + L-Arg Mean ± SEM (Range)	Δ	%Δ	P Level
MDA (µmol/L)	16.8 ± 1.6 (13.6 – 30.4)	13.2 ± 1.1 (8.6 – 26.4)	- 3.6 ± 0.3	- 22.3 ± 2.1	< 0.001
CAT (µmol/ml)	1.7 ± 0.04 (1.4 – 2.0)	2.2 ± 0.06 (1.9 – 2.6)	0.5 ± 0.5	30.9 ± 2.5	< 0.001
SOD (µmol/ml)	23.1 ± 1.2 (13.7 – 33.8)	26.4 ± 1.2 (18.5 – 35.1)	3.3 ± 0.3	15.4 ± 2.0	< 0.001
GP_X (µmol/ml)	1.8 ± 0.1 (0.3 – 3.0)	3.0 ± 0.1 (2.3 - 10.3)	1.1±0.1	86.8 ± 26.0	< 0.01
AST (IU/L)	10.8 ± 1.0 (3.2 – 15.0)	7.2 ± 0.6 (7.4 – 14.4)	-3.6 ± 0.5	-29.0 ± 2.6	< 0.001
ALT (IU/L)	7.1 ± 0.6 (3.2 – 11.8)	4.2 ± 0.3 (2.6 – 10.5)	-2.9 ± 0.5	-35.1 ± 4.2	< 0.001
ALP (IU/L)	278.2 ± 25.1 (148.0–737.0)	117.4 ± 7.3	-160.8± 27.1	-52.6 ± 4.4	< 0.001

Key:
MDA = Malondildehyde; Δ = Change; CAT = Catalase; %Δ = Percent change; SOD = Superoxide dismuthase ALT = Alanine aminotransferase; GPX = Glutathione peroxidase; ALP = Alkaline phosphatase; AST = Aspartate aminotransferase

Table 2 shows that combination therapy (HU + L-Arg) caused an elevation of antioxidant enzymes levels ($p < 0.001$ in each case) but lowered MDA ($p < 0.001$) and each of the liver enzymes levels ($p < 0.001$ in each case).

DISCUSSION

Subjects were paediatric patients of the Paediatric Out-Patients' Sickle Cell Clinic at the Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria. Subjects were in the steady state. Informed oral consent was obtained from a parent of each child. The children had been on hydroxyurea (HU) therapy.

Although BP was not measured before HU therapy, results of this study showed that HU may not have affected the blood pressure of the subjects. The values reported in this study were higher than values reported for normal Nigerian children (Umar *et al*, 2016). Inspite of its benefits, HU is reported not to prevent stroke in SCA sufferers (Atweh and Schetchter, 2001). Blood pressure values in children (Hussain and Hassan, 2017) and adults (Ajayi *et al*, 2013) suffering from SCA had been reported to be lower than in the general population.

Our results show that the combination therapy (HU + Arginine) significantly reduced the blood pressure of the subjects. Morris *et al* (2013) had demonstrated that oral arginine reduced estimated pulmonary artery systolic pressure in sickle cell patients suffering from pulmonary hypertension. That arginine reduced blood pressure when used as an adjunct to hydroxyurea could be regarded as beneficial to the subjects. It had been suggested that blood pressures which appear normal or slightly elevated in healthy individuals may have serious cardiovascular and renal consequences in sickle cell disease patients (Saborio and Scheinman, 1999).

Arginine supplementation following hydroxyurea therapy caused a significant increase in HbF%, [Hb] and PCV. Elevation of foetal haemoglobin in SCD improved the rheological characteristics of erythrocytes by decreasing intracellular polymerization of HbS (Rodgers *et al*, 1993). Hydroxyurea is known to raise the level of HbF and haemoglobin (Agrawal *et al*, 2014). In an earlier study in adult SCD patients, Little *et al*, (2009) could not demonstrate any increase in HbF% when arginine was used as an adjunct to hydroxyurea. Enhancement of HbF%, [Hb] and PCV as seen in this study would improve oxygen delivery to tissues.

Result of this study showed that combining hydroxyurea therapy with L-arginine supplementation decreased blood levels of measured liver enzymes. A comparable study was not found in literature. There is also no report of the effect of hydroxyurea alone on these liver enzymes in sickle cell anaemia. However, L-arginine (Jaja *et al*, 2016) or Vitamin C (Jaja *et al*, 2013) supplementation had been shown to decrease these liver enzymes in adult SCA subjects. Elevated liver enzymes levels in SCA had been related to hepatic damage and haemolysis (Kotila *et al*, 2005; Nsiah *et al*, 2011).

Combined hydroxyurea and l-arginine elevated antioxidant enzymes levels while decreasing lipid peroxidation (MDA). Thus the combination therapy resulted in reduced oxidative stress burden in the patients. This result is similar to that of Little *et al*, (2009) which showed the elevation of glutathione when hydroxyurea was augmented with arginine. The mechanism of action of l-arginine in SCD sufferers had been shown to be by reducing oxidative stress burden through the elevation of antioxidant enzymes levels and reducing malondialdehyde (Kehinde *et al*, 2015; Jaja *et al*, 2020). L-Arginine also elevates trace metals levels in blood which form an integral part of antioxidant enzymes (Ogungbemi *et al*, 2018). It may also act through the nitric oxide pathway (Ogungbemi *et al*, 2013; Bakshi and Morris, 2016; Eleuterio *et al*, 2019). Hydroxyurea, on its own part, inhibits DNA synthesis by inhibiting the activity of ribonucleotide reductase (RR) – the enzyme that converts ribonucleosides into deoxxyribonucleosides which are building blocks for DNA synthesis (Agrawal *et al*, 2014). It also elevates the level of nitric oxide in blood (McGann and Ware, 2015).

The short coming of this study was that basal values of the parameters were not measured before the commencement of hydroxyurea therapy. A more structured study will overcome that shortcoming. In conclusion, the study suggests that a combination of l-arginine and hydroxyurea in the management of paediatric patients suffering from SCA may be beneficial.

Acknowledgements
This study was supported by University of Lagos Grant, CRC 2008/05. We gratefully acknowledge the cooperation of the subjects and their parents.

REFERENCES

Agrawal, R. K., Patel, R. K., Shah, V., Nainiwal, L. and Trivedi, B. (2014). Hydroxyurea in sickle cell disease: Drug Review. Indian J. Hematol. Blood Transfus. 30(2): 91–96.

Ajayi, I. O., Nwokocha, C. R. and Ebeigbe, A. B. (2013). Blood pressure variations in subjects with different haemoglobin genotypes. J. Afr. Ass. Physiol. Sci. 1: 23-26.

Atweh, G. F. and Schechter, A.N. (2001). Pharmacological induction of fetal hemoglobin: raising the therapeutic bar in sickle cell disease. Curr. Opin. Hematol. 8: 123–130.

Bakshi, N. and Morris, C. R. (2016). The role of the arginine metabolome in pain: implications for sickle cell disease. J. Pain Res. 9: 167–175

Carden, M. A. and Little, J. (2019). Emerging disease-modifying therapies for sickle cell disease. Haematologica 104(9): 1710-1719.

Dacie, J. V. and Lewis SM. (1991). Practical Hematology. 7th Ed Edinburgh, Churchill Livingstone.

Diallo, D. and Tchernia, G. (2002). Sickle cell disease in Africa. Curr. Opin. Hematol. 9: 111-116

Eleuterio, R. M. N., Nascimento, F. O., Araújo, T. G., Castro, M. F., Almeida-Filho, T.P., Maia-Filho, P. A., Eleuterio, J., Elias, D. B. and Lemes, R. P. (2019). Double-blind clinical trial of arginine supplementation in the treatment of adult patients with sickle cell anaemia. Adv. Hematol. Article ID 4397150, 6 pages.

Ellman, G. (1959). Tissues sulphydryl groups. Arch. Biochem. Biophysics 82: 70-77.

Hebbel, R. P., Vercellotti, G. M. and Natth, K. A. (2009). A system biology consideration of the vasculopathy of sickle cell anaemia: the need for multi-modality chemoprophylaxis. Cardiovasc. Hematol. Disord. Drug Targets 9: 271–292.

Hussain, A. A. and Hassan, M. K. (2017). Blood pressure of children and adolescents with sickle cell anemia in Basra. Iraq Iranian J. blood & Cancer 9: 101-107.

Ingram, V. M. (1956). A specific chemical difference between the globins of normal human and sickle-cell anemia haemoglobin. Nature 178: 792–794.

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 sickle cell anaemia. Pathophysiol. 23: 81–85.

Jaja, S. I., Kehinde, M. O., Olowoyeye, O. A., Shoneye, K.O., Tubi, O. O. and Adekunle, O. M. (2013). Vitamin C increases catalase but decreases liver enzymes and lipid peroxidation in sickle cell anemia subjects in the steady state. Nig. Qt. J. Hosp. Med. 23: 232-236.

Jaja S.I., Saka, W.A., Ogungbemi, S.I., Anigbogu C.N. and Kehinde M.O. (2020). L-Arginine ameliorates insulin resistance in sickle cell anaemia in the steady state. J. Afr. Ass. Physiol. Sci. 8 (1): 1-7.

Kehinde, M.O., Ogungbemi, S.I., Anigbogu, C.N., Jaja, S.I. (2015). L-Arginine supplementation enhances antioxidant activity and erythrocyte integrity in sickle cell anaemia subjects. Pathophysiol. 22: 137–142.

Kotila, T., Adedapo, K., Adedapo, A., Oluwasola, O., Fakunle, E. and Brown B. (2005). Liver dysfunction in steady state sickle cell disease, Ann. Hepatol. 4: 261–263.

Little, J. A., Hauser, K. P., Martyr, S.E., Harris, A., Maric, I., Morris, C.R., Suh, J. H., Taylor, J., Castro, O., Machado, R., Kato, J. and Gladwin, M. T. (2009). Hematologic, biochemical, and cardiopulmonary effects of l-arginine supplementation or phosphodiesterase 5 inhibition in patients with sickle cell disease who are on hydroxyurea therapy. Eur. J. Haematol. 82: 315–321.

McGann, P. T. and Ware, R. E. (2015). Hydroxyurea therapy for sickle cell anemia. Expert Opin. Drug Saf. 14(11): 1749 – 1758.

Morris, C. R., Morris, S. M., Hagar, W., van Warmerdam, J., Claster, S., Kepka-Lenhart D., Machado, L., Kuypers, F. A. and Vichinsky, E. P. (2003). Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? Am. J. Resp. Crit. Care Med. 168: 63–69.

Nsiah, K., Dzogbefia, V. P., Ansong, D., Akoto, A. O., Boateng, H. and Ocloo, D. (2011). Pattern of AST and ALT changes in relation to hemolysis in sickle cell disease. Clin. Med. Insight Blood Disord. 4: 1–9.

Ogungbemi, S. I., Anigbogu, C. N., Kehinde, M. O. and Jaja, S. I. (2013). L-arginine increases nitric oxide and attenuates pressor and heart rate responses to change in posture in sickle cell anemia subjects. Niger. J. Physiol. Sci. 28: 045 –050.

Ogungbemi, S.I., Jaja, S.I., Anigbogu, C.N., and Kehinde, M.O. (2018). L-arginine enhances blood trace metals and reduces oxidative stress burden in sickle cell anaemia subjects in the steady state. J. Afr. Ass. Physiol. Sci. 6: 145-152.

Pauling, L., Itano, H. A., Singer, S.J. and Wells, I. C. (1949). Sickle cell anemia a molecular disease. Science 110: 543-548.

Piel, F. B., Patil, A. P., Howes, R.E., Nyangiri, O. A., Gething, P. E., Dewi, M., Temperley, W. H., Williams, T. N., Weatherall, D. J. and Hay, S. I. (2013). Global epidemiology of sickle haemoglobin in neonates: a contemporary geosttistical model-based map and population estimates. Lancet 381: 142–151.

Rodgers, G. P., Dover, G. J., Uyesaka, N., Noguchi, C. T., Schechter, A.N. and Nienhuis, A.W. (1993). Augmentation by erythropoietin of the fetal-hemoglobin response to hydroxyurea in sickle cell disease. New Eng. J. Med. 328: 73–80.

Rukkumani, R., Aruna, K., Varma, P. S., Rajasekaran, K.N . and Menon, V. P. (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. J. Pharm. Pharmaceutical Sci. 7: 274-283.

Saborio, P. and Scheinman, J. I. (1999). Sickle cell nephropathy. J. Am. Soc. Nephrol. 10: 187-192.

Titus, J., Chari, S., Gupta, M. and Parekh, N. (2004). Pro-oxidant and anti-oxidant status in patients of sickle cell anemia. Ind. J. Clin. Biochem. 19: 168-172.

Umar, A., Mustafa, A. and Muuta, I. (2016). Prevalence of elevated blood pressure among primary school children in Kano Metropolis, Nigeria. Nig. J. Cardiol. 13: 57-61.

Niger. J. Physiol. Sci. 35 (December 2020): 135 - 142
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Intestinal Glucose Release Following Insulin-Induced Hypoglycemia in Dogs: Implication of Gluconeogenesis and Glycogenolysis

***Shittu S.T., Alada A.R.A. and Oyebola D.D.O.**

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

Summary: This study was designed to investigate the source of the glucose released by the small intestine during insulin-induced hypoglycaemia in dogs. Experiments were carried out on fasted, male, anaesthetized mongrel dogs divided into 3 groups (n = 5 each). Group 1 received normal saline (0.2 ml/kg) and served as the control while groups 2-3 were injected with low (5 i.u/kg) and high (8 i.u/kg) doses of insulin. The left femoral artery and vein were cannulated for arterial blood sampling and intravenous administration of normal saline or insulin, respectively. Through a midline laparatomy, a vein draining the upper jejunum was cannulated for Intestinal Blood Flow (IBF) measurement and jejunal venous blood sampling. In stabilized animals, basal measurement of IBF and levels of glucose and lactate in blood were obtained prior to the injections and monitored for 90 minutes post injection. Intestinal Glucose/Lactate Uptake was calculated as the product of IBF and arterio-venous glucose /lactate difference. Jejunal tissue samples were obtained for the determination of Glycogen Content and activities of glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase. Data are presented as Mean ± SEM and compared by student's t-test and ANOVA. Intestinal blood flow was significantly increased by insulin. Within 20 minutes post injection of insulin, glucose uptake was negative while lactate uptake increased. Glycogen content, glycogen synthase activity and hexokinase activity were significantly reduced in the insulin groups compared with the control while glycogen phosphorylase 'a' and glucose-6-phosphatase activities were increased significantly. In conclusion, the glucose released during insulin-induced hypoglycemia may receive inputs from the breaking down of glycogen and synthesis of glucose within the small intestine.

Keywords*: Glucose release, lactate uptake, Glycogen metabolism, Jejunum, Dogs*

**Address for correspondence: st.shittu@ui.edu.ng; Tel: +234-8052644680*

Manuscript Accepted: November, 2020

INTRODUCTION

Previous studies have shown conclusively that the gastro-intestinal tract plays a modulatory role in glucose homeostasis in dogs (Alada and Oyebola, 1996; Alada *et al*., 2005; Salman *et al*., 2014; Shittu *et al*., 2018). Thus, when the blood glucose was increased following hyperglycaemia induced by adrenaline (Grayson and Oyebola, 1983; Alada and Oyebola, 1996, Oyebola *et al*., 2011), glucagon (Alada and Oyebola 1996; 1997), nicotine (Grayson and Oyebola, 1985; Oyebola *et al*., 2009), infusion of glucose and other sugars (Alada and Oyebola 1996; Salman *et al*., 2014) or by hyperglycaemia that occurs in diabetes mellitus (Alada *et al*; 2005), the intestine significantly increased its glucose uptake by as much as 700 to 1000 %. Also, when the blood glucose was reduced, for instance, by administration of insulin to hypoglycaemic level, the intestine pushes out glucose into blood circulation (Alada and Oyebola, 1996; Alada *et al*., 2005). Interestingly, a reduction in blood glucose above the hypoglycaemic levels as reported by Alada *et al*. (2005) in dogs did not cause a release of glucose from the intestine. Thus, the intestinal glucose release is dependent on the presence of hypoglycemia.

Recently, Shittu *et al*. (2018) provided strong evidence to suggest that the huge amount of glucose taken up by the intestine in response to the induced hyperglycaemia was mostly converted to glycogen and to some extent oxidized by glycolysis, judging by the increased levels of lactate in circulation. However, the metabolic source of the glucose that is released into circulation in response to insulin-induced hypoglycaemia (Alada and Oyebola, 1996; 1997) is still not known. For example, in the liver, which is the major blood glucose regulating organ in the body, glucose is released into circulation through the processes of gluconeogenesis and glycogenolysis because the liver possesses the enzyme glucose-6-phosphatase which is a key enzyme involved in the last biochemical step leading to the production of glucose in gluconeogenesis and glycogenolysis. Mitheux *et al*. (2004) reported the expression of glucose-6-phosphatase gene in the intestine of rat and man, thereby suggesting that the intestine of both rat and man could also be involved in endogenous glucose production. With the use of molecular techniques and monitoring gene expression of glucose-6-phosphatase, Penhoat *et al*. (2014) were able to show that during fasting, the small intestine of mice also contributes significantly to endogenous glucose production. It was therefore, concluded that under certain conditions such as fasting and hypoglycaemia, the small intestine of rat, mouse and man behaves essentially like the liver and kidney in regulation of glucose homeostasis. For instance, the endogenous glucose production of the small intestine could increase from 5 – 10 % in post-absorptive rat to 20 – 25 % after 24hr of fasting (Croset *et al*., 2001; Mithieux *et al*., 2006). It must however,

be noted that the metabolic pathways for endogenous glucose production in the liver is not the same as in the small intestine (Penhoat *et al*., 2014).

The present study was therefore designed to investigate the possible source of glucose that is released into circulation following insulin-induced hypoglycaemia in dogs.

MATERIALS AND METHODS

The experimental procedure was as earlier described (Shittu *et al*., 2018). Adult male mongrel dogs (9 – 11 kg) were anaesthetized (Sodium Pentobarbitone, 30 mg/kg) after an overnight fast prior to the commencement of the experiments. They were allowed to breathe at room temperature via a Y-piece cannula inserted into the trachea. The left femoral artery and vein were cannulated with the arterial canula advanced to the level of the superior mesenteric artery for arterial sampling while the venous canula was for drug delivery. A midline laparotomy was carried out to isolate the jejunum, then a vein draining the proximal segment of the jejunum was cannulated for jejunal venous blood sampling. The jejunal vein cannula was moved into an extra-corporeal position and a non-crushing clamp was applied to its free end. Intravascular blood clotting was prevented by administration of Sodium heparin (300 I.U/kg, i.v) and supplemental doses of the anaesthetic agent were given as required. By two layered interrupted sutures, the abdomen was closed and the dogs were allowed to stabilize for 60 minutes.

Experimental procedure: The experiments were carried out on three groups (control and insulin groups) with 5 dogs per group. Basal measurements of jejunal blood flow, arterial and venous glucose and lactate levels were done in the stabilized animals. Low (5 i.u/kg) or high doses (8 i.u/kg) of insulin (Actrapid®, Norvo Nordisk) was then injected intravenously and the variables were monitored for 90 minutes at 5 minutes interval post- injection of insulin. Timed collection was used to determine jejunal blood flow (Alada and Oyebola, 1996). Arterial and Venous glucose and lactate levels were determined using glucose oxidase and lactate dehydrogenase methods, respectively; arterio-venous differences (A-V) were calculated for glucose [$(A\text{-}V)_{glucose}$] and lactate [$(A\text{-}V)_{lactate}$]. Intestinal Glucose Uptake (IGU) and lactate uptake were calculated as the product of intestinal blood flow and [$(A\text{-}V)_{glucose}$] or [$(A\text{-}V)_{lactate}$]. Jejunal tissue biopsy was taken 10-15 minutes post injection for glycogen content determination and for the assays of glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase enzymes activities. The effect of normal saline (0.2 ml/kg) on the measured variables was studied in another 5 dogs and this served as the control group.

Determination of jejunal glycogen content: The method of Seifter *et al.* (1950) as modified by Jermyn (1975) was used to quantify jejunal glycogen content. The tissue was digested in potassium hydroxide, washed with ethanol, centrifuged and drained. The obtained white precipitate was reconstituted in water and step-wisely reacted with hydrochloric acid, formic acid and anthrone reagent. It was incubated at 100 ^{0}C for 10 minutes, cooled and the absorbance of the blue colored solution was taken at 630 nm. The glycogen concentration (mg/ml) was read from a glycogen standard curve. Glycogen content/100 g tissue was calculated as detailed earlier (Shittu *et al*., 2018).

Tissue homogenization for enzyme activities: The tissues were first rinsed in ice cold 1.15% KCl, freed of intestinal contents and dabbed on filter paper. Samples for glycogen synthase, glycogen phosphorylase and hexokinase activities were homogenized in 0.05 M phosphate buffer saline (pH 7.4) while samples for glucose-6-phosphatase activity were homogenized in sucrose buffer containing 0.25 M sucrose, 4 mM EDTA and 1 mM NaF. Protein contents of the supernatants were determined by Biuret method.

Determination of jejunal glycogen synthase activity: Jejunal glycogen synthase activity was assayed using the spectrophotometric stop rate (Kinetic) method of Danforth (1965). Briefly, 100 µl of tissue supernatant was added to a reaction cocktail containing 0.5M Tris HCl Buffer (pH 8.2), $MgCl_2$, EDTA-tetrasodium, β-Mercaptoethanol, UDPG, glycogen and deionized water. It was mixed by inversion and incubate for 5 minutes at 30 ^{0}C. The reaction was stopped by heating the test or blank for 5 minutes at 100 ^{0}C then cool over running tap water. The solutions were transferred into eppendorf tubes and centrifuged. The obtained supernatant (100 µl) was added into another reaction cocktail containing 0.2M Tris HCl Buffer (pH 7.5), KCl, $MgSO_4$, Phosphoenol pyruvate, EDTA-tetrasodium, β-NADH, deionized water and PK/LDH enzyme suspension. It was mixed immediately by inversion and the decrease in absorbance was recorded for five minutes. The final absorbance was obtained for both test and blank supernatant.

Glycogen synthase (Units/mg. protein)

$$= \frac{\Delta \text{ AbsorbanceTest} - \Delta \text{ Absorbance Blank } (2.91)}{(5)(6.22)(0.1)(protein\ concentration)}$$

Determination of jejunal glycogen phosphorylase 'a' activity: Glycogen phosphorylase 'a' activity was assayed using the spectrophotometric stop rate (Kinetic) method of Fischer *et al*. (1962) and Bergmeyer *et al.* (1974). Briefly, in the absence of 5´-AMP, 100 µl of jejunal tissue supernatant was added to a reaction cocktail containing 0.5M Potassium Phosphate Buffer, Glycogen, $MgCl_2$, EDTA, phosphoglucomutase and NADPH, pH 6.8 at 30 ^{0}C. It was mixed by inversion and increase in absorbance was monitored at 340nm for approximately 10 minutes and the ΔA_{340}/minute was obtained for both the Test and Blank. The Phosphorylase a units/ml enzyme was calculated as:

$$\frac{\left(\Delta\frac{\text{Absorbance}}{\text{min}}\text{Test without 5´AMP} - \Delta\frac{\text{Absorbance}}{\text{min}}\text{Blank without 5´AMP}\right)(2.95)(df)}{(6.22)(0.1)}$$

Where df is the diluting factor

The Phosphorylase a units/mg protein

$$= \frac{\text{Phosphorylase a units/ml enzyme}}{protein\ concentration}$$

Determination of Hexokinase activity: Hexokinase activity in the jejunum was determined by the method described by Branstrup *et al.* (1957) wherein the rate of disappearance of glucose was determined at 38 ^{0}C in a buffer solution containing ATP, Magnesium, KCl and

Fluoride. Briefly, 2 ml of Glucose buffer [0.0025 M glucose, 0.0025 M $MgCl_2$, 0.025 M NaF, 0.01 M K_2HP0_4, 0.077 M KCl, and 0.03 M Tris (Hydroxy-methyl) aminomethane, (Trizma base) pH 8] was pipetted into a test tube followed by 0.1 ml of 0.18 M ATP solution and 0.9 ml of distilled water. The mixture was preheated in water for 5 minutes at 38 ^{0}C, 1 ml of jejunal homogenate was added and 100 µl of the homogenate-buffer substrate mixture was taken immediately for initial glucose analysis. The mixture was then incubated at 38 ^{0}C for 30 minutes and another 100 µl was taken for final glucose analysis. The difference in the level of glucose was calculated and hexokinase activity was expressed as glucose metabolised/mg. pr/30min. All assays were carried out in duplicates. In this assay, glucose was assayed using a commercially available Glucose GOD-PAP kit (Fortress Diagnostic®, United Kingdom).

Determination of Glucose-6-Phosphatase activity: Glucose-6-phosphatase activity was assayed according to the method of Koide and Oda (1959) based on the principle that the enzyme acts as phosphohydrolase and phosphotransferase. The reaction involves the formation of covalently bound enzyme-inorganic phosphate intermediate that can liberate inorganic phosphate in the presence of an acceptor. The liberated inorganic phosphate can then be quantified using a suitable method. Briefly, into a test tube, 0.3 ml of 0.1 M citrate buffer (pH 6.5), 0.5 ml of 150 mM glucose-6-phosphate solution and 0.2 ml of sucrose buffer extracted jejunal homogenate were mixed and incubated at 37 ^{0}C for 1 hour. At the end of the incubation period, 1.0 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction and placed on ice. After 10 minutes on ice, the mixtures were centrifuged. Aliquot (1 ml) of the supernatant was then used for the determination of liberated phosphate by the method of Fiske and Subbarow (1925).

Statistical Analysis

Data were presented as Mean± SEM of the variables measured. Differences in mean values were compared using student' t-test and ANOVA. P values of 0.05 or less were taken as statistically significant.

RESULTS

Effect of insulin on arterial and venous blood glucose level: The effects of insulin on arterial and venous blood glucose levels are shown in Table 1. The two doses of insulin administered to the dog produced immediate reduction in blood glucose level which was sustained throughout the post-injection observation period. At low dose of insulin, the arterial blood glucose decreased from a basal value of 106.4 ± 4.69 mg/dl to a minimum value of 91.8 ± 1.59 mg/dl. When high dose of insulin was administered, the arterial blood glucose level further decreased from 106.2 ± 4.48 mg/dl to 52.20 ± 7.28 mg/dl.

Insulin administration also reduced significantly the venous blood glucose level. However, between 5 to 20 minutes post-injection, the venous blood glucose was higher than the arterial blood glucose level.

Effect of insulin on intestinal blood flow, arterio-venous glucose difference and intestinal glucose uptake: The effects of insulin on intestinal blood flow is shown in figure 1(A). Insulin produced a significant increase in intestinal blood flow within the first 15 minutes post-injection which declined gradually to basal value in the remaining part of the post-injection observation period. Intestinal blood flow increased from basal value of 10.4 ± 0.35 ml/min to a maximum value of 11. 8 ± 0.37 ml/min in response to low dose of insulin and further increased from 10.6 ± 0.24 ml/min to 12.5 ± 0.43 ml/min in response to high dose of insulin. There was however no difference in the increased intestinal blood flow produced by the two doses of insulin. The effect of insulin on arterio-venous glucose difference is shown in figure 1(B). Insulin caused negative arterio-venous glucose difference in the first twenty minutes post-injection of insulin and thereafter increased towards basal value in the remaining part of the post-injection observation period. Low dose decreases arterio-venous glucose difference from a basal value of 2.8 ± 0.73 mg/dl to a minimum value of -4.40 ± 1.94 mg/dl. High dose of insulin had a more profound effect on arterio-venous glucose difference, for instance, arterio-venous glucose difference decreased from the basal value of 3.00 ± 0.55 mg/dl to the minimum value of -6.80 ± 1.28 mg/dl in response to high dose of insulin.

The effect of insulin on intestinal glucose uptake is shown in figure 1(C). Insulin significantly produced negative intestinal glucose uptake between 5 minutes to 20 minutes post injection and returned to values that is not different from the basal in the remaining part of the observation period. In other words, insulin pushes out glucose in to circulation between 5-20 minutes post-injection. Low dose of insulin produced decreased intestinal glucose uptake from a basal value of 29.2 ± 8.17 mg/min to -49.0 ± 10.86 mg/min. while high dose of insulin decreased intestinal glucose uptake from a basal value of 29.8 ± 5.76 mg/min to -82.0 ± 14.02 mg/min. The percentage decrease in intestinal glucose uptake is about 275%.

Table 1.

Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on arterial and venous glucose level in dogs (n=5). *P<0.05, **P<0.01, ***P<0.001.

	Dose	0 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	75 min	90 min
Arterial Glucose level (mg/dl)	Low Dose	106.4 ±4.69	102.6 ±2.73	97.4 ±2.52	91.8 ±1.59**	94.8 ±3.74*	93.4 ±2.80*	96.4 ±2.94*	94 ±1.87*	94.6 ±4.65	95.4 ±2.50
	High Dose	106.20 ±4.48	82.60 ±4.29***	52.20 ±7.28***	55.40 ±8.13***	70.60 ±5.03***	90.80 ±3.82**	100.00 ±3.33*	102.80 ±3.32	98.00 ±2.07	103.00 ±5.18
Venous Glucose level (mg/dl)	Low Dose	103.6 ±5.32	103.4 ±3.12	99 ±2.84	96.2 ±1.85	95.2 ±3.74	91.2 ±2.55*	93.6 ±3.00	90.8 ±1.88*	92.1 ±4.48	92.6 ±2.67
	High Dose	103.30 ±4.13	94.60 ±6.24**	59.00 ±7.46**	57.80 ±8.66**	72.20 ±5.75**	88.20 ±4.20**	97.40 ±3.36*	99.40 ±3.21	93.80 ±2.13	100.40 ±5.45

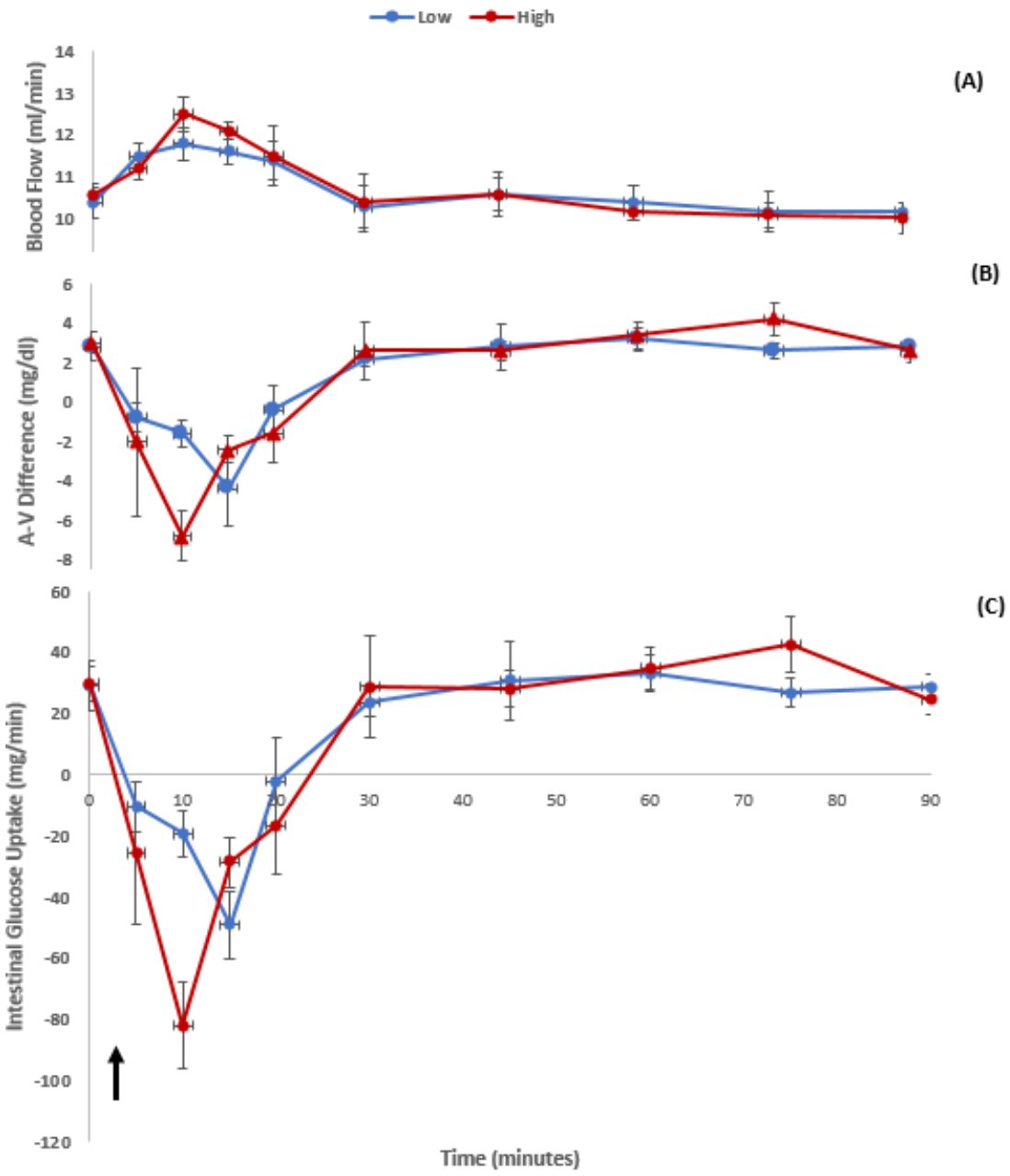


Figure 1.
Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on (A) jejunal blood flow, (B) Arterio-venous glucose difference and (C) Intestinal glucose uptake in dogs (n=5). Black arrow indicates point of injection.

Table 2.
Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on arterial and venous lactate level in dogs (n=5). *P<0.05.

	Dose	0 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	75 min	90 min
Arterial Lactate level (mg/dl)	Low dose	20.60 ±1.90	21.03 ±1.93	22.97 ±1.00	25.62 ±1.64*	26.42 ±2.64*	27.648 ±2.12*	28.02 ±3.37*	26.93 ±2.78*	29.05 ±2.92*	31.09 ±3.22*
	High dose	22.22 ±2.67	25.71 ±2.65	27.38 ±2.05*	26.43 ±2.98	25.91 ±2.82	25.16 ±3.26	27.36 ±2.19*	25.54 ±3.08	29.52 ±2.51*	27.78 ±3.28
Venous lactate level (mg/dl)	Low dose	24.05 ±2.25	19.62 ±1.70*	21.62 ±2.40	22.09 ±1.37	25.09 ±2.36	26.71 ±2.42	28.98 ±3.24	28.47 ±3.55	31.92 ±3.10	33.71 ±2.08
	High	26.36 ±1.64	22.92 ±1.32*	21.86 ±1.71*	25.68 ±1.84	26.52 ±2.27	27.25 ±1.81	28.85 ±1.45	28.18 ±2.05	31.04 ±2.40*	31.03 ±2.87*

Effect of insulin on arterial and venous blood lactate level: The effects of insulin on arterial and venous blood lactate levels are shown in Table 2. Insulin caused an immediate increase in arterial blood lactate level which was sustained throughout the post-injection observation period. Venous lactate level was transiently decreased between 5 to 15 minutes post-injection of insulin and thereafter increased throughout the remaining part of the post-injection observation period.

Hypoglycemia induces intestinal glycogenolysis and gluconeogenesis

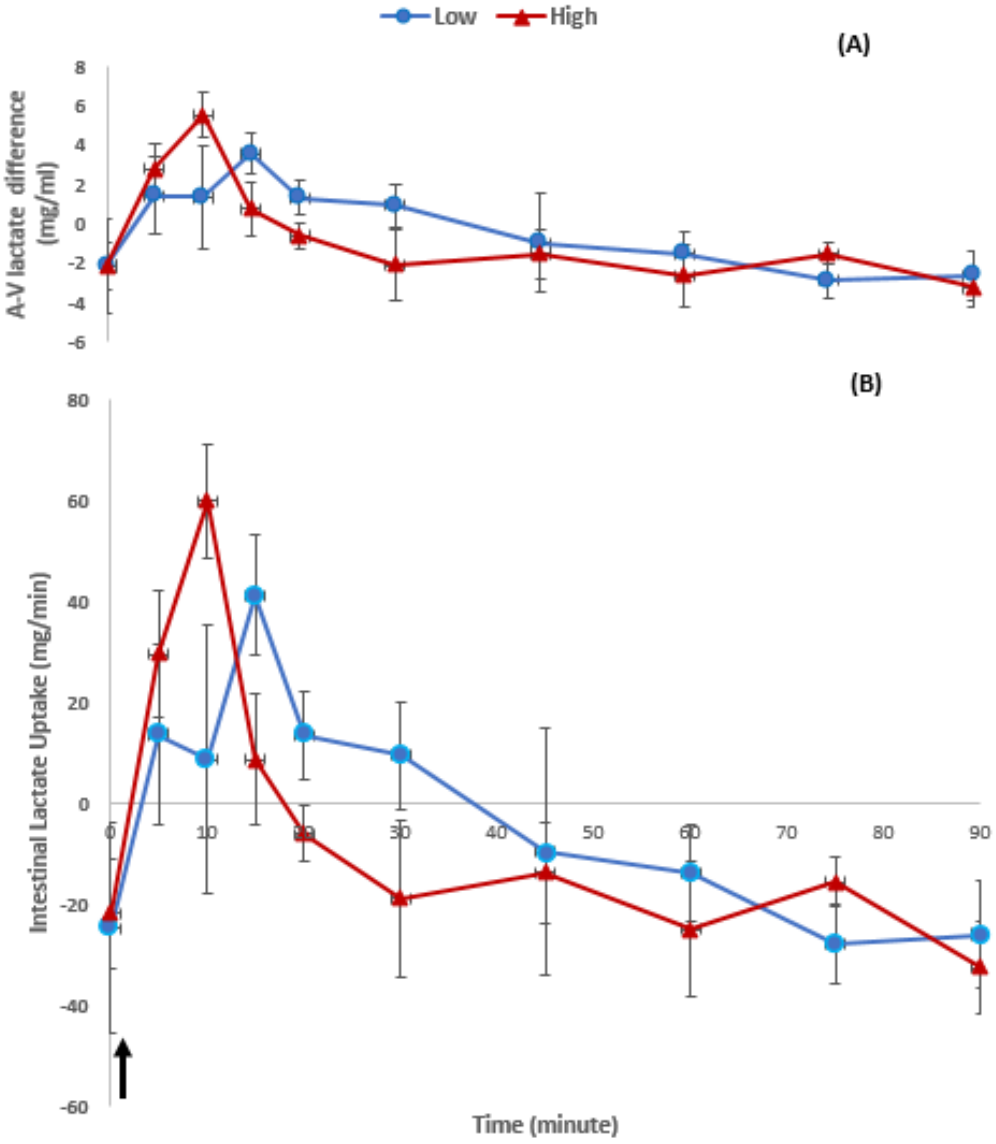


Figure 2.
Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on (A) Arterio-venous (A-V) lactate difference and (B) intestinal lactate uptake in dogs (n=5). Black arrow indicates point of injection.

Effect of insulin on arterio-venous lactate difference and intestinal lactate uptake: The effect of insulin on arterio-venous lactate difference is shown in figure 2(A). Insulin injection produced a positive arterio-venous lactate difference between 5 minutes to 15 minutes post-injection. In other words, arterio-venous lactate difference which hitherto was negative was reversed by insulin injection within 5-15 minutes post-injection and gradually became negative till the end of the observation period. For instance, following low dose, arterio-venous lactate difference increased from a basal value of -2.14 ± 2.41 mg/dl to a peak value of 3.53 ± 1.04 mg/dl at 15minutes post injection. High dose insulin produced a more profound effect on arterio-venous lactate difference which increased from a basal value of -2.14 ± 1.23 mg/dl to a peak value of 5.54 ± 1.14 mg/dl at 10 minutes post-injection.

The effect of insulin on intestinal lactate uptake is shown in figure 2(B). Insulin reversed the negative intestinal lactate uptake between 5 minutes to 15 minutes post injection which became negative again at 20 minutes till the end of the observation period. That is to say that insulin produced a positive intestinal lactate uptake which indicates that the intestine actually extracted lactate from the blood stream between 5 to 15 minutes following insulin injection. Low dose increased intestinal lactate uptake from basal value of -24.64 ± 20.87 mg/min to a peak significant value of 41.35 ± 11.85 mg/min at 15 minutes post-injection while high dose increased intestinal lactate uptake from -21.76 ± 10.96 mg/min to 60.09 ± 11.36 mg/min at 10 minutes post-injection. The percentage increase in intestinal lactate uptake for low dose and high dose are 168% and 276%, respectively.

Effect of insulin on intestinal glycogen content
The effect of insulin on intestinal glycogen content is shown in Figure 3. Intestinal glycogen content decreased from 138.72 ± 4.58 mg/100g to 106.90 ± 2.29 mg/100g and 97.39 ± 4.33 mg/100g following low and high dose insulin

injection these represented a 23% and 30% depletion following injection of low and high dose of insulin, respectively. There was no significant difference in the depletion of intestinal glycogen content caused by the two doses of insulin.

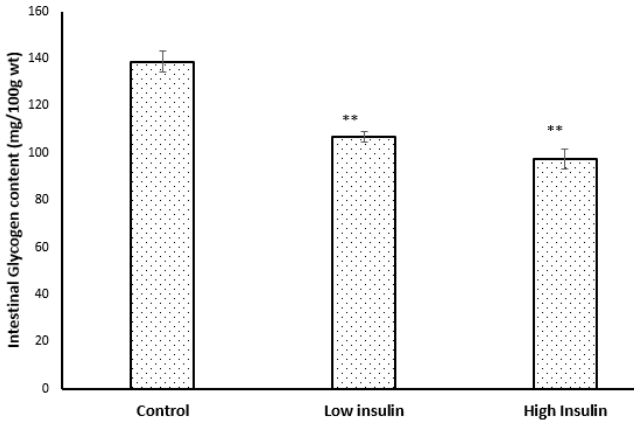


Figure 3:
Effect intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on intestinal glycogen content (n=5). **P<0.01.

Table 3.
Effect intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on intestinal glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase activities

Enzyme activity	Control	Low Dose	High Dose
Glycogen Synthase (Activity/mg.pr)	1.29 ± 0.13	0.79 ± 0.13**	0.41 ± 0.11**#
Glycogen Phosphorylase a (x 10^{-3}Activity/mg.pr)	1.74 ± 0.21	5.14 ± 0.45**	6.58 ± 0.18**#
Hexokinase (Activity/mg.pr)	1.28 ± 0.20	0.34 ± 0.02*	0.26 ± 0.03**#
Glucose 6-Phosphatase (Activity/mg.pr)	30.71 ± 1.56	45.73 ± 3.10**	41.87 ± 2.72**

Effect of insulin on intestinal glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase activities

The effect of insulin on the intestinal enzyme activities are shown in Table 3. Insulin decreased intestinal glycogen synthase activity in a dose-dependent manner. In other words, high dose of insulin produced a more profound effect on the activities of glycogen synthase. For example, intestinal glycogen synthase activity decreased from 1.29 ± 0.13 activity/mg.pr to 0.79 ± 0.13 activity/mg.pr for low dose of insulin and 0.41 ± 0.11 activity/mg.pr for high dose of insulin.

The two doses of insulin significantly increase glycogen phosphorylase 'a' activity in a dose-dependent manner. Glycogen phosphorylase 'a' activity increased from 1.74 ± 0.21 activity/mg.pr 5.14 ± 0.45 activity/mg.pr and 6.58 ± 0.18 activity/mg.pr for low dose and high dose of insulin respectively.

Hexokinase activity significantly decreased from 1.28 ± 0.20 activity/mg.pr to 0.34 ± 0.02 activity/mg.pr and 0.26 ± 0.03 activity/mg.pr for the low dose and high dose of insulin respectively. On the other hand, glucose-6-phosphatase activity increases from 30.71 ± 1.56 activity/mg.pr to 45.73 ± 3.01 activity/mg.pr and 41.87 ± 1.56 activity/mg.pr. There was however, no difference in the increase in glucose-6-phosphatase activity induced by the two doses of insulin.

DISCUSSION

The observed increase in the blood flow through the intestinal mesenteric veins is consistent with reports in previous studies in rats (Grayson and Kinnear, 1958) and dogs (Grayson and Mendel, 1965; Alada and Oyebola, 1996). This increase occurs irrespective of blood pressure change (Alada and Oyebola, 1996). There are however reports that the rise in blood flow is not a direct effect of insulin *per se* on intestinal vessels but rather secondary to stimulation of sympathetic system and release of catecholamines (Alada and Oyebola, 1997).

The hypoglycaemic action of insulin is well- known. Insulin acts through activation of tyrosine kinase in the beta subunit and phosphorylating several proteins to increase glucose uptake essentially in the liver, skeletal muscles and adipose tissue (Kasuga *et al*., 1983, Yamauchi *et al*., 1996)

The most relevant results in this study is the negative intestinal glucose uptake observed within the first twenty minutes post-injection of insulin when the arterio-venous glucose balance across the intestinal segment was negative. In other words, the intestine was actually involved in endogenous glucose production in dogs during the insulin-induced hypoglycaemia. Similar observations have been reported in rat and humans (Mitheux *et al*., 2004, Rajas *et al*., 2009) and mouse (Penhoat *et al*., 2014) in studies employing tracer techniques and gas chromatography in determining arterio-venous glucose balance. Previous workers (Mitheux *et al*., 2001; Rajas *et al*., 2001) have also provided strong evidence to show that the small intestine is involved in endogenous glucose production through gluconeogenesis. Indeed, gene expression of major gluconeogenic enzymes, including glucose-6-phosphatase, pyruvate carboxylase and phosphoenol carboxykinase have been reported in the small intestine of rat and human (Croset *et al*., 2001; Mithieux *et al*., 2004). Mithieux *et al*. (2004) reported that the small intestine produces glucose using glutamine and glycerol as the main precursors. Abundance of the enzyme glutaminase was also reported in the small intestine of rat and human (Mithieux *et al*., 2005).

Interestingly, intestinal gluconeogenesis was reported to occur only during 24 to 48 hours fasting and under diabetic condition and this could account for as high as 30% of endogenous glucose production in the body (Penhoat *et al*., 2014). There have been other reports of intestinal gluconeogenesis following hepatectomy-induced hypoglycaemia (Battezatti *et al*., 2004, Penhoat *et al*., 2014). In the present study, hypoglycaemia was induced by a single intravenous injection of insulin at both low and high doses. A similar observation of intestinal glucose release was reported in an earlier study (Alada *et al*., 2005) with diabetic dogs following significant reduction of diabetic hyperglycaemia with insulin injections.

The observed increase in intestinal lactate uptake following a rise in arterial blood lactate level in this study is noteworthy. The increase in intestinal lactate uptake also corresponds in timing with the negative arterio-venous glucose balance of the intestine. In other words, while there was an increase in the uptake of lactate by the intestine, glucose was also being released into circulation by the intestine. Although Mitheux and his co-workers (2004) have reported that in rat and human, glutamine and glycerol are the main metabolic precursors for the formation of glucose in the intestinal gluconeogenesis, while lactate is the major precursor in the liver and the kidney. To the best of our knowledge, there has been no report of intestinal gluconeogenesis in the dog. However, even in those animals where there was evidence of intestinal gluconeogenesis, there are still doubts on the metabolic pathways involved in the endogenous glucose production. In this study, an observation of the presence of and a significant increase in the activity of glucose-6-phosphatase in the intestine of dog give support to the hypothesis that the intestine of dog is most probably also involved in gluconeogenesis as earlier reported for rat (Croset *et al*., 2001), mouse (Penhoat *et al*., 2014) and human (Mithieux *et al.*, 1999). Further studies using molecular techniques and knockout model may throw more light on this observation.

Another possible source of the glucose released from the intestine following insulin-induced hypoglycaemia in this study is through glycogenolysis. Some workers (Rajas *et al*., 1999; Mithieux *et al*., 2004) had earlier reported on intestinal glycogenolysis during hypoglycaemia in rat and human. The observed decrease in intestinal glycogen content and activity of intestinal glycogen synthase with concomitant increase in activity of intestinal phosphorylase 'a' following insulin-induced hypoglycaemia in this study is consistent with the possibility of intestinal glycogenolysis in dogs. The observed increase in the activity of glucose-6-phosphatase in this study also supports the possibility of intestinal glycogenolysis since the enzyme is important in breaking down glucose-6-phosphate into glucose and phosphate ion.

In conclusion, this study provided evidence to show that the intestine of dog could be involved in gluconeogenesis and glycogenolysis, thereby accounting for the source of the glucose released into circulation following insulin-induced hypoglycaemia. However, more studies employing tracer and molecular techniques with knockout animal models may be able to provide more information on our observations.

REFERENCES

Alada, A. R. A. and Oyebola, D. D. O. (1996). Evidence that the gastrointestinal tract is involved in glucose homeostasis. Afr. J. Med. And Med. Scr. 25: 243 – 249.

Alada, A. R. A. and Oyebola, D. D. O. (1997). The Role of Adrenergic Receptors in the increased glucose uptake by canine gut. Afr. J. Med. & Med. Sci. 26: 75 – 78.

Alada, A. R. A., Falokun, P. O. and Oyebola, D. D. O. (2005). Intestinal glucose uptake in normal, untreated and insulin –treated diabetic dogs. Afr. J. Med. & Med. Sci. 34, 147-156

Battezzati, A., Caumo, A., Martino, F., Sereni, L.P., Coppa, J., Romito, R., Ammatuna, M., Regalia, E., Matthews, D.E., Mazzaferro, V., and Luzi, L. (2004). Nonhepatic glucose production in humans. Am. J. Physiol. Endocrinol. Metab. 286, E129–E135.

Bergmeyer, H. U, Gawehn K. and Grassi M. (1974). Glycogen Phosphorylase activity. In Bergmeyer H. U. edited Methods of Enzymatic Analysis, 2nd edition, Vol 1, page 505-507, Academic Press inc, New York

Branstrup, N., Kirk J. E. and Bruni C. (1957). Hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individual of various ages. J Gerentol 12: 166-170

Croset, M., Rajas, F., Zitoun, C., Hurot, J. M., Montano, S, Mithieux, G. (2001). Rat small intestine in an insulin sensitive gluconeogenic organ. Diabetes, 50:740–746.2.

Danforth W. H. (1965). Glycogen synthetase activity in skeletal muscle. Journal of Biological Chemistry 240, 588-593.

Fischer, E. H and Krebs, E. G. (1962). Methods in Enzymology, Volume 5, 369-373

Fiske, C. H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.

Grayson, J. and Oyebola, D. D. O. (1983). The effect of catecholamines on intestinal glucose and oxygen uptake in dog. J. Physiol (Lond.) 343: 311 – 322.

Grayson, J. and Oyebola, D. D. O. (1985). Effect of nicotine on blood flow, oxygen consumption and glucose uptake in the canine small intestine. Br. J. Pharmacol 85: 797 – 804.

Grayson, J. and Kinnear, T. (1958). Vascular and metabolic responses of the liver to insulin. The Journal of Physiology.144(1):52-67.

Grayson, J. and Mendel, D. (1965). Physiology of the Splanchnic Circulation, p. 106. London: Edward Arnold.

Jermyn, M. A. (1975). Determination of Glycogen. Increasing the sensitivity of the anthrone method for carbohydrate. Analytical Biochem. 68: 322- 335.

Kasuga, M., Y. Fujita-Yamaguchi, D. L. Blithe, and C. R. Kahn. (1983). Tyrosine-specific protein kinase activity is associated with the purified insulin receptor. Proc. Natl. Acad. Sci. USA 80:2137–2141.

Koide, H. and Oda T. (1959). Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. Clin. Chim. Acta, 4: 554-561.

Mithieux, G., Rajas, F. and Gautier-Stein, A. (2004). A novel role for glucose-6 phosphatase in the small intestine in the control of glucose homeostasis. J Biol Chem. 279:44231–44234.

Mithieux, G., Misery, P., Magnan, C., Pillot, B., Gautier-Stein, A., Bernard, C., Rajas, F. and Zitoun, C. (2005). Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein. Cell Metabolism, 2, 321–329.

Mithieux, G., Gautier-Stein, A., Rajas, F., and Zitoun, C. (2006). Contribution of intestine and kidney to glucose fluxes in different nutritional states in rat. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 143, 195–200.

Mithieux, G., Andreelli, F. and Magnan, C. (2009). Intestinal gluconeogenesis: key signal of central control of energy and glucose homeostasis. Current Opinion in Clinical Nutrition and Metabolic Care 12:419–423.

Oyebola, D. D. O., Idolor, G. O, Taiwo, E. O., Alada A. R. A., Owoeye O. and Isehunwa G. O. (2009). Effect of

nicotine on glucose uptake in the rabbit small intestine. Afr. J. Med. And Med. Sci.38: 119 – 130

Oyebola, D. D. O., Taiwo, E. O., Idolor, G. O. and Alada, A. R. A. (2011). Effect of adrenaline on glucose uptake in the rabbit small intestine. Afr. J. Med. And Med. Sci. 40, 225-233.

Penhoat, A., Fayard, L., Stefanutti, A., Mithieux, G., and Rajas, F. (2014). Intestinal gluconeogenesis is crucial to maintain a physiological fasting glycemia in the absence of hepatic glucose production in mice. Metabolism 63, 104–111

Rajas, F., Bruni, N., Montano, S., Zitoun, C. and Mithieux, G. (1999). The glucose-6 phosphatase gene is expressed in human and rat small intestine: regulation of expression in fasted and diabetic rats. Gastroenterology, 117, 132–139.

Rajas, F., Croset, M., Zitoun, C., Montano, S., and Mithieux, G. (2000). Induction of PEPCK gene expression in insulinopenia in rat small intestine. Diabetes 49, 1165–1168

Salman, T. M., Alada, A. R. A and Oyebola, D. D. O. (2014). Intestinal glucose uptake responses to infusion of glucose, fructose and galactose in dogs. Niger. J. Physiol. Sci. 29(2), 023 –027.

Seifter, S., Dayton, S., Novic, B. and Muntwyler, E. (1950). The estimation of glycogen with the anthrone reagent. Arch. Biochem. 25: 191-200

Shittu S.T, Alada A.R.A and Oyebola D.D.O (2018). Metabolic Fate of Glucose Taken up by the Intestine During Induced Hyperglycaemia in Dogs. Nigerian Journal of Physiological Sci. 33 (1): 037-049

Yamauchi, T., Tobe, K., Tamemoto, H., Ueki, K., Kaburagi, Y., Yamamoto-Honda, R., Takahashi, Y., Yoshizawa, F., Aizawa, S., Akanuma, Y., Sonenberg, N., Yazaki, Y., Kadowaki, T. (1996). Insulin signalling and insulin actions in the muscles and livers of insulin-resistant, insulin receptor substrate 1-deficient mice. Molecular and Cellular Biology 16 (6): 3074–3084.

Niger. J. Physiol. Sci. 35 (December 2020): 143 - 146
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Comparative Efficacy of Soft Tissue Massage and Transcutaneous Electric Nerve Stimulation in the Management of Hemiplegic Shoulder Pain

***Badaru U.M. and Aminu S**

Department of Physiotherapy, Faculty of Allied Health Sciences, Bayero University, Kano, Nigeria

Summary: Hemiplegic Shoulder pain (HSP) is a common clinical consequence of focal cerebral insult. The study investigated the comparative efficacy of Transcutaneous Electrical Nerve Stimulation (TENS) and Soft Tissue Massage (STM) in the management HSP. A total of 50 consenting stroke patients with HSP completed the 8 weeks pretest posttest quasi-experimental study. They were assigned into either TENS or STM groups using simple random sampling. Both TENS and STM treatments were administered on subscapularis, supraspinatus and posterior deltoid muscles for 16 sessions. HSP was evaluated pre and post intervention with visual analog scale. Within and between group differences in HSP were compared using paired and unpaired t-tests respectively with SPSS version 16.0 with probability level of 0.05 to indicate level of significance. The age of patients in the TENS and STM groups was 56±9.26years and 57±7.51years respectively. Duration of stroke was 10±6 months and 9±4 months for TENS and STM groups respectively. There was no significant between group differences in HSP at baseline (TENS=4.76±2.17; STM=5.48±2.06; p>0.05). Within group comparison of HSP scores pre and post intervention in the TENS group indicated a significant reduction (P<0.05); also the same applies to STM group (P<0.05). When the post treatment HPS scores were compared across the groups, there was a significant difference in favor of TENS group (TENS=1.48±0.51; STM=2.12±1.17; p<0.05). Both TENS and STM contribute to the modulation of HSP in stroke patients and each could become handy in augmenting other forms of management. However, TENS is more effective.

Keywords*: Hemiplegic shoulder pain, TENS, Soft Tissue Massage, Subscapularis, Deltoid, Supraspinatus*

**Address for correspondence: umbadaru.pth@buk.edu.ng ; Tel: +234-8035913759*

Manuscript Accepted: November, 2020

INTRODUCTION

Shoulder pain has negative impact on the discharge of economic, family and social roles (Yeun, 2017). Hemiplegic shoulder pain (HSP) hampers physical function (Joshi and Chitra, 2017) and negatively influenced the outcome of stroke rehabilitation (Walsh, 2001; Poenaru *et al*, 2008; Joshi and Chitra, 2017). It occurs commonly 2-3 months post stroke (Poduri 1993). Common causes of HSP include poor handling and positioning of the affected upper limb (Walsh, 2001), joint subluxation due to flaccidity of rotator cuff muscles (Yu *et al* 2004; Teasell *et al*, 2012) and spasticity (Yu *et al*, 2004; Paci *et al*, 2005; Poenaru *et al*, 2008; Chuang *et al*, 2017; Zhou *et al*, 2018).

Transcutaneous Electric Nerve Stimulation (TENS) is a physiotherapy modality that is frequently used in the management of HSP. It has been reported being effective in relieving HSP (Leandri *et al*, 1990; Ekim *et al*, 2008; Poenaru *et al*, 2008; Moniruzzaman *et al*, 2010; Joshi and Chitra, 2017) and improving upper extremity function (Poenaru *et al*, 2008; Joshi and Chitra, 2017) especially when administered in conjunction with conventional physiotherapy. Some studies have however found that TENS was less effective than other forms of therapy such as neuromuscular electrical stimulation (NMES) (Chuang *et al*, 2017; Zhou *et al*, 2018) and manual tapping technique (Tiwari *et al*, 2018) while the study by de Jong *et al*, (2013) and Bello and Amaezo (2009) reported respectively that NMES and TENS are not effective in HSP modulation. The conflicting report on the efficacy of TENS was due largely to the great variation that exist in the technique of TENS application on the shoulder and in the choice of the shoulder muscles to be treated. Soft Tissue Massage (STM) is another modality commonly used by physiotherapists to manage HSP (Karels *et al*, 2006; Bennell *et al*, 2007, Bello and Amaezo, 2009). A recent systematic review suggested that STM is effective in reducing shoulder pain from causes other than stroke (Yeun, 2017). Todate only the study by Mok and Woo, (2004) shows that STM significantly reduced HSP hence further research is needed to provide recommendations that are based on empirical evidence. This study therefore investigated the comparative efficacy of TENS and STM in the management HSP with both treatments applied on subscapularis, supraspinatus and posterior deltoid muscles..

MATERIALS AND METHODS

The pretest posttest quasi-experimental comparative study was carried out at the departments of physiotherapy of Aminu Kano Teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital (MMSH), Kano. Ethical approval was obtained from the ethics committees of AKTH and the Kano State Hospital Management Board for

MMSH. A total of 56 participants met the inclusion criteria; they were consecutively recruited and randomly assigned into the 2 groups as they become available. Participants that met the inclusion criteria include all stroke patients diagnosed with HSP with the exception of those with insensate skin, diagnosis of shoulder subluxation, pre-stroke history of frozen shoulder, trauma or brachial plexus injury and those who were presently on prescribed pain medications. The aims, objectives and study procedures were explained to each of the participants. Thereafter they were asked to give written informed consent of participation which they all did. The consent included beneficence, voluntariness, confidentiality and anonymity which were duly respected.

Procedure for Random Assignment into Treatment Groups: Each of the patients was asked to pick a single paper without replacement from a bowl containing a mixture of 56 folded papers that were thoroughly mixed together (letter 'A' was written on 50% of the folded papers while letter 'B' was written on the remaining 50%). Each patient was assigned to the group he/she picked. Participants who picked letter 'A' were assigned to the TENS group while those who picked letter 'B' were the STM group. Visual analog scale was used to assess level of HSP at baseline and post intervention. TENS unit (Enraf Nonius, Vamed Engineering, Serial No-PE0007-KN-005) was used for treatment of HSP. The STM was done manually with olive oil being used as massage medium. Methylated Spirit was used to sterilize the shoulder areas to be treated.

Assessment of Level of Hemiplegic Shoulder Pain: This was assessed at baseline and post intervention periods by a blinded assessor using the visual analog scale. The participants were asked to rate their level of shoulder pain from 'no pain' or score of zero (0) to 'worst pain' or score of ten (10).

Range of Motion Exercise: All the study participants regardless of their group assignment received both passive and active range of motion exercises to the affected shoulder complex for 5 minutes pre and post TENS or STM treatments.

Procedure for TENS Treatment: Participants in the TENS therapy group were asked to shave their axillar. Each of the patients was asked to assume side lying posture on the unaffected side. Low frequency TENS (frequency, 80HZ, phase duration 60μs) was applied for 10 minutes on each of subscapularis posterior deltoid and supraspinatus muscles twice weekly x 8 weeks. Each of the 2 TENS electrodes was placed on either end of the supraspinatus muscle. They were thereafter, placed along the length of the posterior deltoid muscle. Similar technique for determination of the bellies of supraspinatus and posterior deltoid muscles and electrode placements was reported in recent study (Chuang *et al*. 2017). Furthermore, one of the electrodes was placed in the origin of the subscapular nerve in the neck and while the other one was placed in the posterior wall of the axilla as contained in Gulick *et al*, (2007) for the stimulation of subscapularis muscle. In the study by Thurner *et al*, (2013) therapy targeting the subscapularis muscle belly was administered on the posterior wall of the axilla with the patient's shoulder in abduction. The TENS therapy session lasted 40 minutes (each of the 3 muscles received 10 minutes of TENS with 5 minutes of ROM exercises before and after TENS treatment)

Procedures for STM: The patients in the STM group were asked to assumed side lying posture on the unaffected side. The bellies of the specific muscles to be treated (subscapularis, posterior deltoid and supraspinatus) were identified in the similar way it was done for electrode placement. Slow stroking STM was administered directly on posterior deltoid and supraspinatus muscles for 10minutes each twice weekly for 8weeks. However because of the hidden nature of the subscapularis muscle (it is located between scapular and thorax), the STM was done indirectly by the gentle gliding of scapular on the thoracic wall for 10 minutes twice weekly for 8weeks.
Each STM session lasted 40 minutes (each of the 3 muscles had STM for 10 minutes in addition to 5 minutes of ROM exercises before and after STM). Participants in both groups were asked to continue with their routine gait training, however any form of exercise or therapy to the shoulder joint was not allowed, participants only received TENS or STM in addition to ROM for treatment of their HSP during the period of the study.

Data Analysis Procedure: Demographic characteristics of the study participants were analyzed using descriptive statistics of mean and standard deviation. Both within and between group differences were analysed using inferential statistics of paired and independent sample t-test respectively. All statistical analysis was performed using statistical package for social sciences (SPSS) version 16.0 with probability level of 0.05 to indicate level of significance

RESULTS

The 50 participants that completed the study comprised TENS Group with 25 patients, 14 males (56%) and 11 females (44%) while the 25 patients in the STM group comprised 15 males (60%) and 10 females (40%). The mean age of participants in the TENS group was 56years, while that of STM group was 57years as presented in Table1.

Table1.
Physical Characteristics of Stroke Patients (N=50)

VARIABLES		GROUP A	GROUP B
Age (years)		56± 9.26	57±7.51
Duration of stroke (months)		10±6	9±4
		n (%)	**n (%)**
Gender	Male	14(56)	15 (60)
	Female	11(44)	10 (40)
	Total	25(100)	25(100)
Occupation		**n (%)**	**n (%)**
	Unemployed	11(44)	10(40)
	Self-employed	5(20)	8(32)
	Civil-servant	9(36)	7(28)
	Total	25(100)	25(100)
Side of hemiplegia		**n (%)**	**n (%)**
	Right	15(60)	11(44)
	Left	10 (40)	14(56)
	Total	25(100)	25(100)

SD=Standard Deviation; N=Sample Size; n=Frequency; %=Percent

Table 2.
Between Group Comparison of HSP Scores at Baseline and Post Intervention Periods

Variables	N	Mean ± SD	df	t	P
Baseline					
TENS Group	25	4.76 ± 2.17	48	-1.20	0.24
STM Group	25	5.48 ± 2.06			
Post intervention					
TENS Group	25	1.48 ± 0.51	48	-2.51	0.02*****
STM Group	25	2.12 ± 1.17			

*SD=standard deviation; df=degree of freedom *=Significant; TENS=Transcutaneous Electric Nerve Stimulation; STM=Soft Tissue Massage*

Table 3.
Within-Group Comparison of HSP Scores Pre and Post Interventions in TENS and STM Groups

Variables	N	Mean ± SD	df	t	P
Group A					
HSP Pre-intervention	25	4.76 ± 2.17	24	-1.20	0.000*
HSP Post-intervention	25	1.48 ± 0.51			
Post intervention					
HSP Pre-intervention	25	5.48 ± 2.06	24	-2.51	7.40
HSP Post-intervention	25	2.12 ± 1.17			

*N= no of participants; M=mean; SD=standard deviation; df = degree of freedom *significant*

Between-group comparison of HSP at baseline using independent samples t-test showed insignificant difference in HSP scores before the interventions (P>0.05). At the end of 8 weeks of intervention, there was significant difference in HSP between the 2 groups with the TENS group having significantly lower pain scores (P<0.05) as presented in table 2. The result of within-group comparisons of pre and post intervention scores of HSP in STM group was significant (P<0.05). Furthermore, the result of within-group comparison of pain scores pre and post intervention periods in the TENS group was also significant (P<0.05). These results are presented in Table 3.

DISCUSSION

This study compared the effectiveness of TENS and STM in the management of HSP with both therapies being applied on supraspinatus, posterior deltoid and subscapularis muscles. The result this study showed that TENS has significant effect on HSP and this implies that TENS therapy can be used to effectively reduce HSP. The outcome of this study as regards the effectiveness of TENS in the management of HSP is in consonance with the reports of other studies where it was equally reported being effective in reducing HSP and improving functional use of the affected upper extremity especially when used in conjunction with conventional physiotherapy (Leandri *et al*, 1990; Ekim *et al*, 2008; Poenaru *et al*, 2008; Moniruzzaman *et al*, 2010; Joshi and Chitra, 2017). The outcome of this study was however different from that of Bello and Amaezo, (2009) who found on the other hand that TENS therapy was not effective in the management of HSP probably due to the fact that TENS was to treat the entire shoulder joint. In the present study, however, the TENS therapy targeted specific muscles that stabilize the glenohumeral joint and the exact points of TENS application has been clearly highlighted.

It was further found in the present study that STM significantly reduced HSP. This implies that STM can potentially be used to reduce shoulder pain post stroke. The finding of the present study on STM is in consonance with the reports of Mok and Woo (2004) which showed that slow-stroking STM significantly reduced HSP. The possible reason for the similarity between our finding and that of Mok and Woo (2004) could because the type of STM used was similar in both studies. The outcome of this study supported the finding of the systematic review by Yeun, (2017) that STM could effective in reducing shoulder pain from various aetiologies.

Additionally, the present study found that TENS therapy decreased HSP more significantly than STM. This is because participants who received TENS therapy recorded significantly less pain scores at the end of the last treatment. This implies that TENS is more effective than STM in the management of HSP. Similar studies have also reported that TENS was more effective than galvanic current (Poenaru *et al*, 2008) and ultrasonic therapy (Moniruzzaman *et al*, 2010) in the management of HSP. Although some studies have reported that other modalities such as NMES (Chuang *et al*, 2017; Zhou *et al*, 2018) and manual tapping technique (Tiwari *et al*, 2018) were more effective when compared with TENS in the management of HSP, probably because the studies did not include subscapularis as one of the target muscles in the management of HSP. Even in a study in which the subscapularis muscle was the primary target, the exact point of application of therapy on the scapularis muscle was not clearly stated (Poenaru *et al*, 2008). Though supraspinatus and posterior deltoid are important muscles in maintaining the correct alignment and stabilization of the glenohumeral joint (Paci *et al*, 2005; Zhou *et al*, 2018), the subscapularis muscle could be the chief culprit and a major cause HSP that is often overlooked. Being the major internal rotator of the shoulder, the subscapularis is part of the typical flexor synergy in patients with spastic hemiplegia that usually produce pain during shoulder abduction, flexion and external rotation (Teasell *et al*, 2012). Trigger points in the subscapularis muscle may produce increased pain sensitization and significant muscle guarding that restricts mobility of shoulder joint (Thurner *et al*, 2013).

In view of the result obtained from this study, it can be concluded that both TENS and STM therapies can significantly reduce HSP post stroke when the treatment targeted supraspinatus, posterior deltoid and subscapularis muscles. TENS is however more effective than STM in the modulation of HSP when applied in conjunction with ROM exercise.

Based on the results of this study, it was recommended that TENS should be considered the first treatment of choice in management of HSP. Further studies should be conducted to find the efficacy of TENS and STM therapies combined.

REFERENCES

Bello, A.I. and Amedzo, M.Y. (2009). Relative Effectiveness of Transcutaneous Electrical Nerve Stimulation and Hot Packs in the Management of

Hemiplegic Shoulder Pain. J Nig Societ Physiother. 17:1-6

Bennell, K. Coburn, S. and Wee, E. (2007). Efficacy and cost-effectiveness of a physiotherapy program for chronic rotator cuff pathology: a protocol for a randomised, double-blind, placebo-controlled trial. BMC Musculoskeletal Disord. 8:86.

Chuang, L-L. Chen, Y-L. Chen, C-C. Li, Y-C. Wong, A. M-K. Hsu, A-L. *et al*. (2017). Effect of EMG-triggered neuromuscular electrical stimulation with bilateral arm training on hemiplegic shoulder pain and arm function after stroke: a randomized controlled trial. J NeuroEng Rehabil. 14:122.

De Jong, L.D. Dijkstra, P.U. Gerritsen, J. Geurts, A.C.H. and Postema, K. (2013). Combined arm stretch positioning and neuromuscular electrical stimulation during rehabilitation does not improve range of motion, shoulder pain or function in patients after stroke: a randomised trial. J Physiother. 59:245-254

Ekim, A. Armağan, O. and Oner, C. (2008). Efficiency of TENS treatment in hemiplegic shoulder pain: a placebo controlled study. Agri. 20(1):41-6.

Gulick, D.T. Borger, A. and McNamee, L. (2007). Effect of analgesic nerve block electrical stimulation in a patient with adhesive capsulitis. Physiother Theory Pract. 23(1):5763

Joshi, D. and Chitra, J. (2017). Effect of scapular proprioceptive neuromuscular facilitation on shoulder pain, range of motion, and upper extremity function in hemiplegic patients: A randomized controlled trial. Indian J Health Sci Biomed Res. 10:276-82

Karels, C. Polling, W. and Bierma-Zeinstra, S. (2006). Treatment of arm, neck, and/or shoulder complaints in physical therapy practice. Spine. 2006; 31: 584–9.

Leandri, M. Parodi, C.I. and Corrieri, N.(1990). Comparison of TENS treatments in hemiplegic shoulder pain. Scand J Rehabil Med 1990; 22: 69–71

Mok E, Woo CP. The effects of slow-stroke back massage on anxiety and shoulder pain in elderly stroke patients. Complement Ther Nurs Midwifery. 2004;10: 209-216.

Moniruzzaman M, Salek KM, Shakoor MA, Mia BA, Moyeenuzzaman M (2010): Effects of therapeutic modalities on patients with post stroke shoulder pain. Mymensingh Med J. 19(1):48- 53.

Paci, M. Nannetti, L. and Rinaldi, L.A. (2005). Glenohumeral subluxation in hemiplegia: an overview. J Rehabil Res Dev 2005;42:557–568

Poduri, K.R. (1993). Shoulder pain in stroke patients and its effects on rehabilitation. J Stroke Cerebrovasc Dis. 3: 261-266.

Poenaru, D. Cinteza, D. Popescu, S. Ionita, L. and Mateescu, M. (2008). Shoulder pain management in stroke. Mædica- J Clin Med. 3 (3): 162-167

Teasell, R. Foley, N. and Bhogal, S.K. (2012). Painful Hemiplegic Shoulder. The Evidence- Based Review of Stroke Rehabilitation (EBRSR) reviews current practices in stroke rehabilitation. Accessed at www.ebrsr.com

Thurner, M.S. Donatelli, R.A. and Bascharon, R. (2013). Subscapularis Syndrome: A Case Report. Int J Sports Phys Ther. 8(6): 871

Tiwari, M. Saini, M. and Goswami, Y. (2018). A Comparative Study on Effectiveness of Taping and TENS on a Painful Shoulder in Patient with Acute Stroke. J Yoga Physiother. 2018; 4(3): 555-637.

Walsh K. (2001). Management of shoulder pain in patients with Stroke. Postgrad Med J. 77:645–649

Yeun, Y-R. (2017). Effectiveness of massage therapy for shoulder pain: a systematic review and meta-analysis. J Phys Ther Sci. 29: 936–940

Yu, D.T. Chae, J. Walker, M.E. Kristeins, A. Elovic, F.P. and Flanagan, S.R. (2004). 'Intramuscular neuromuscular electric stimulation for post stroke shoulder pain: a multicentre randomised clinical trial', Arch Phys Med Rehabil. 85(5):695-704.

Zhou, M. Li, F. Lu, W. Wu, J. and Pei, S. (2018). Efficiency of Neuromuscular Electrical Stimulation and Transcutaneous Nerve Stimulation on Hemiplegic Shoulder Pain: A Prospective Randomized Controlled Trial. Arch Phys Med Rehabil. 2018; 99(9):1730-1739.

Niger. J. Physiol. Sci. 35 (December 2020): 147 - 153
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Dose-dependent changes in Haematological and Serum Biochemical Variables in Male Wistar Rats Exposed to Sodium Metavanadate'

Omayone T.P[1,2]., Salami A.T.[2], Odukanmi O.A.[2], Olopade J.O[3]. and *Olaleye S.B.[2]

[1]*Department of Physiology, School of Health and Health Technology, Federal University of Technology Akure, Akure, Nigeria.*

[2]*Gastrointestinal Secretion and Inflammation Research Unit, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria.*

[3]*Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria.*

Summary: The interest in the role of vanadium compounds in living organisms has grown tremendously especially since the report of its glycemic normalization activity in the 1980s. There has been reports of both its toxic as well as positive effects, thus there is a paucity of information on the essentiality of this element in biological systems. In this study, the effect of different doses of sodium metavanadate on the haematological and biochemical variables of male Wistar rats was investigated. Twenty male Wistar rats were divided into four groups of five each and were given tap water containing various concentrations of sodium metavanadate (0ppm- group 1, 50ppm- group 2, 100ppm- group 3, or 200ppm- group 4) for 10weeks. Weekly body changes were noted and blood was collected at the end of 10 weeks by retro orbital puncture for haematological and serum biochemical variables. Histological sections were also performed on liver and kidney tissues. There was a significant increase in body weight in the 50ppm group compared with control. Sodium metavanadate at 200ppm caused a significant decrease in packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and Lymphocytes with significant increases in neutrophils and neutrophil-lymphocyte ratio when compared with control values. There was also a significant decrease in ALP, ALT and a significant increase in urea concentration in the 200ppm group when compared with control values. All doses of sodium metavanadate significantly reduced blood glucose level. Sections of liver and kidney revealed severed damage at 200ppm compared with control. The results from this study showed that vanadium affects both haematological and biochemical parameters and could be toxic at higher concentrations, while at low concentration could be beneficial as seen with the enhanced body weight.

Keywords*: Vanadium, Haematology, Serum biochemicals, Body weight, Toxicity, Wistar rats*

**Address for correspondence: sbolaleye@yahoo.com; Tel: +234-8023255893*

Manuscript received: March, 2020
Revised version accepted: August 2020

INTRODUCTION

Vanadium as a heavy metal has gained considerable attention over the past few years due to its increase in concentration in the environment yearly, leading to high concentrations in the atmosphere, soil and in water bodies as well as it is increased used as a dietary supplement for body building (Lin *et al*., 2004). The prevalence of vanadium exceeds that of such well-known metals as copper and lead, and equals that of zinc and tin. In a study conducted by Wogu and Okaka, (2011) in Warri River, Delta state Nigeria, vanadium content ranges from 0-0.26mg/L collected at different sites of the river, and was seen to be higher than the concentration of cadmium, chromium and lead in the same water. Vanadium compounds exist in over 50 different mineral ores at concentrations of between 10 and 4100ppm and in association with fossil fuels, particularly coal (at concentrations of between 19 and 126ppm in ash) and crude oil (at concentrations of between 3 and 257ppm) (Gummow, 2011). The average worldwide soil levels of vanadium have been reported to be approximately 100mg/kg (IPCS, 2001).

Vanadium like other heavy metals cannot be destroyed through biological degradation, thus it accumulates in the protoplasm of organism, both plants and animals (Marcano *et al*., 2006). It has long been known that vanadium is toxic to both man and animals. However, the pathogenesis of vanadium poisoning is still poorly understood. There are varying reports on the effects of vanadium to human health as some authors have reported its toxicity on body systems especially on the respiratory, nervous and on haematological parameters (Dabros *et al*., 2006; Worle-Knirsch *et al*., 2007; Ngwa *et al*., 2009; Olopade *et al*., 2011). However, there are also reports on its therapeutic effect in the management of certain diseases such as diabetes (Francik *et al*., 2011; Saima, 2013), osteoporosis in diabetes model (Barrio and Etcheverry, 2006; Sanchez-Gonzalez *et al*., 2017), cancer (Das *et al*., 2012) as well as in gastric ulcer (Kemeir, 2013; Omayone *et al*., 2016). It can therefore be seen that the essentiality as well as the toxic effect of vanadium to humans is still poorly understood. The effect of vanadium is dependent on mode of administration, concentration and duration of exposure. Most of the reports on vanadium toxicity are based on exposure via inhalation,

while reports on oral exposure have shown little or no toxicity. With its dual effect, the need to determine an effective as well as a toxic concentration of vanadium exposure orally therefore necessitates this study.

MATERIALS AND METHODS

Chemical: Vanadium in the form of sodium metavanadate ($NaVO_3$) was purchased from BDH Chemicals Ltd Poole England product.

Animals and treatment: Twenty male albino rats of Wistar strain weighing between 90-120g were used for the study. Animals were obtained from the Central Animal House, College of Medicine University of Ibadan, and were exposed to food and water *ad libitum*. Animals were randomly divided into four groups of five animals each and exposed to various concentration of vanadium (0ppm-control, 50, 100 and 200 ppm) in their drinking water for 10 weeks. Blood samples were collected by retro orbital puncture after 10 weeks of exposure as described by Hoff, (2000) into an Ethylene-diamine-tetra-acetic acid (EDTA) bottle. The blood was then analyzed for haematological and biochemical parameters. Liver and kidney were excised and fixed in 10% formalin for histological assessment.

Haematological Analysis: The haematolotical studies were perform on packed cell volume, Haemoglobin levels, white blood cell count (WBC), Red blood cell count (RBC), Platelets, Lymphocytes, Neutrophils, Monocytes, Eosinophils. This was done according to the method of Dacie and Lewis 1994.

Biochemical Analysis: Plasma levels of levels of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Glucose, Creatinine, Urea and Cholesterol were measured by a colorimetric method using commercial kits (Randox laboratories limited, United Kingdom). Plasma protein was determined by the method of Gornal *et al*., (1949).

Statistical analysis: All values are presented as mean ± SEM and were analysed using One-way ANOVA. The statistical difference was taken to be significant at $p<0.05$.

RESULTS

Body weight gain: The administration of sodium metavanadate at 50 ppm for 10 weeks significantly increase body weight of animals beginning from the 6th week compared with the control group ($p < 0.05$). The body weight gain for other vanadium treated groups showed no significant difference compared with control group. The result is shown in Fig 1.

Haematological variables in blood after sodium metavanadate exposure: Sodium metavanadate showed a dose dependent decrease in PCV, HB, RBC count (Figures 2, 3 and 4 respectively) which was only significant at 200 ppm compared with control. White blood cell and Platelet counts (Figures 5 and 6 respectively) were also decreased in a dose dependent manner and was significant at 100 and 200 ppm compared with control.
Lymphocyte count (Figure 7) was significantly decreased in 200 ppm group, while a significant increase neutrophil count (Figure 8) as well as neutrophil-lymphocyte ratio (NLR) (Figure 9) was noticed in 200ppm group compared with control group at $p<0.05$. The 50ppm group showed no significant changes in all the parameters compared to the control group.

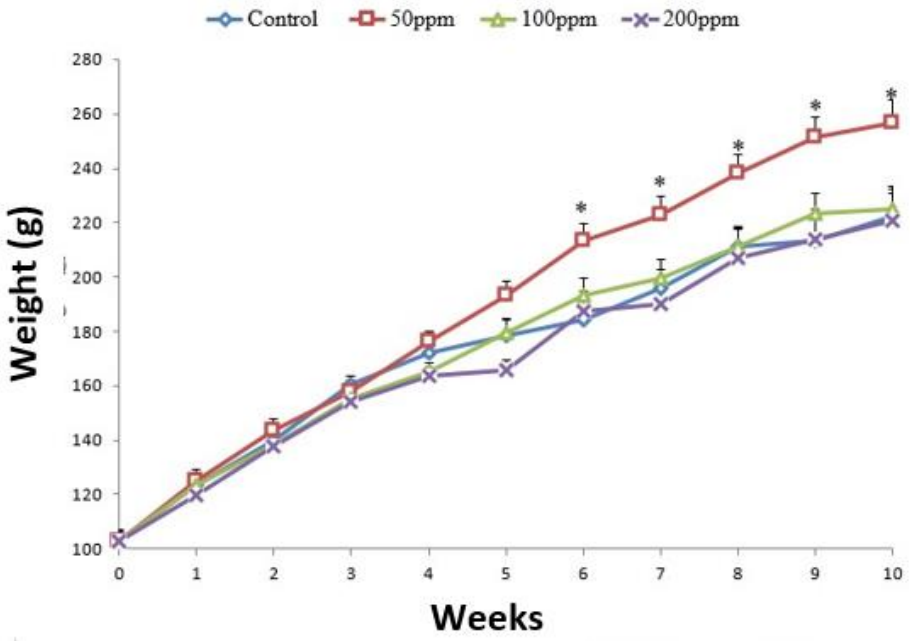


Figure 1:
Effect of Vanadium on body weight after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control*.

Effect of vanadium on hematological and serum biochemical variables

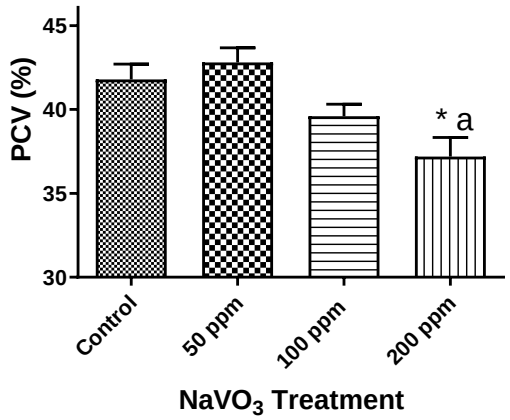


Figure 2:
Effect of Vanadium on PCV after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control.*
a Significant at p<0.05 when compared with 50 ppm.

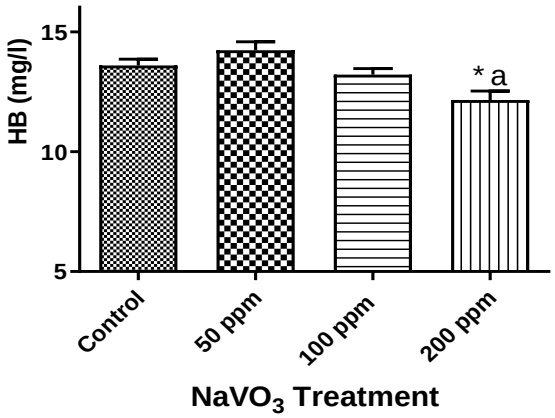


Figure 3:
Effect of Vanadium on HB after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control*
a Significant at p<0.05 when compared with 50 ppm.

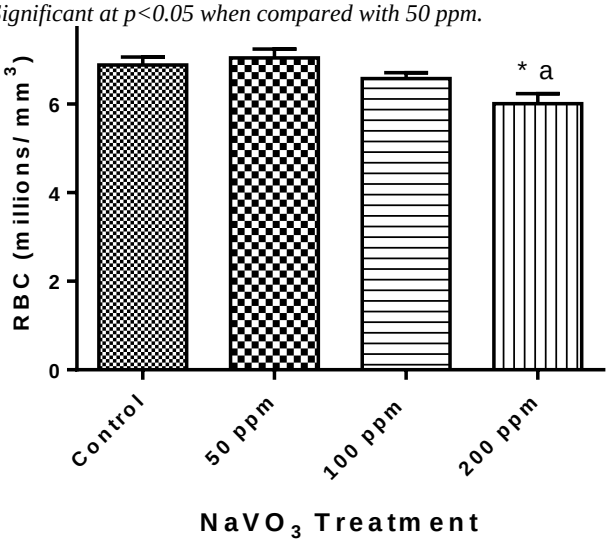


Figure 4:
Effect of Vanadium on RBC after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control.*
a Significant at p<0.05 when compared with 50 ppm.

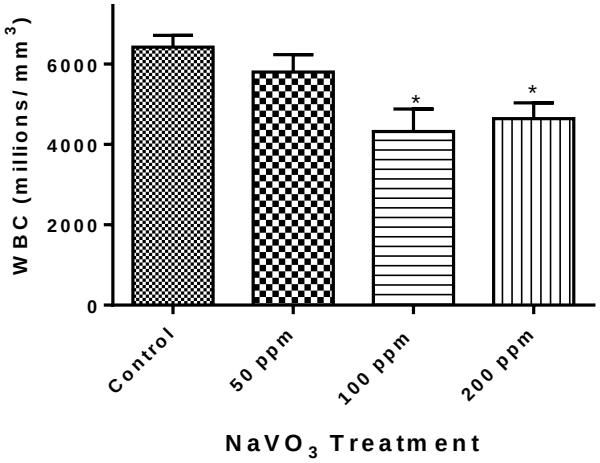


Figure 5:
Effect of Vanadium on WBC after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control*

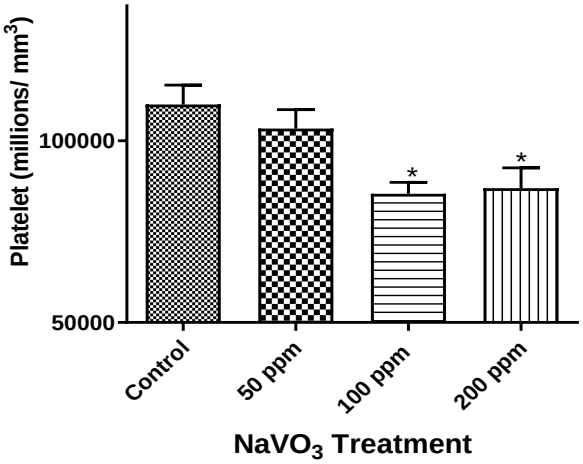


Figure 6:
Effect of Vanadium on Platelet after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control*

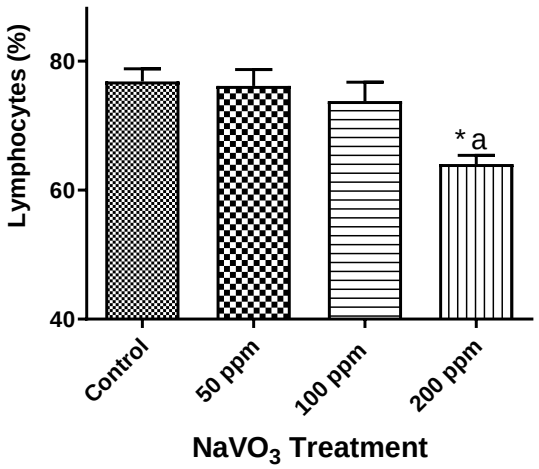


Figure 7:
Effect of Vanadium on lymphocyte after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control*
a Significant at p<0.05 when compared with 50 ppm.

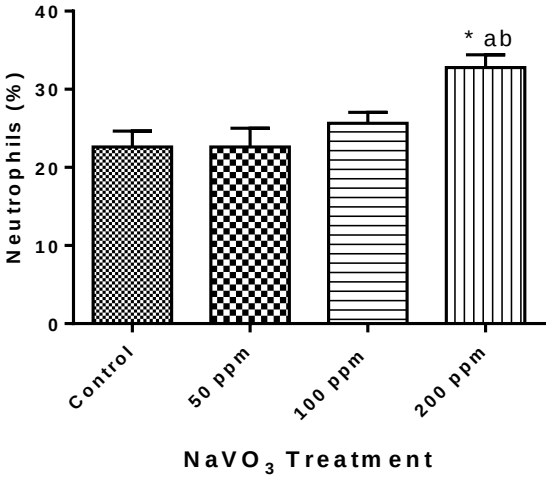


Figure 8:
Effect of Vanadium on neutrophils after 10 weeks exposure
Values are presented as Mean ± SEM, n=5
* Significant at p<0.05 when compared with control
a Significant at p<0.05 when compared with 50 ppm.
b Significant at p<0.05 when compared with 100 ppm.

Changes in plasma biochemical variables after sodium metavanadate exposure: Plasma proteins (globulin and albumin) changes were not significant in all vanadium treated groups compared with control group. Changes in AST were also not significantly different in vanadium treated groups compared with control. ALT and ALP were significantly decreased by 200 ppm sodium metavanadate, while 50 ppm and 100 ppm groups had no significant changes compared with control.

Urea and cholesterol were significantly higher in 100ppm and 200 ppm respectively compared with control group. All vanadium treated groups had significantly lowered plasma glucose level compared with control group, while no significant change was observed in creatinine concentration. The results are presented in Table 1.

Changes in liver and kidney histology after sodium metavanadate exposure: The architecture of liver and kidney were significantly distorted by vanadium at a concentration of 200ppm. Histological sections of the liver and kidney showed blood vessel congestion and severe infiltration of inflammatory cells respectively.

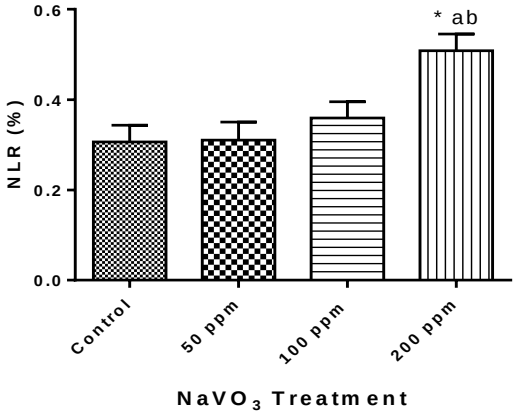


Figure 9:
Effect of Vanadium on neutrophil-lymphocyte ratio after 10 weeks
Values are presented as Mean ± SEM, n=5
** Significant at p<0.05 when compared with control*
a Significant at p<0.05 when compared with 50 ppm.
b Significant at p<0.05 when compared with 100 ppm.

Table 1:
Effect of Sodium metavanadate on some biochemical parameters after 10 weeks exposure.

		Control	50ppm	100ppm	200ppm
Parameters	Albumin	4.88±0.15	4.82±0.17	4.46±0.09	5.0±0.15
	Globulin	3.14±0.02	3.14±0.14	3.16±0.02	3.08±0.14
	Total Protein	8.02±0.15	7.96±0.22	7.62±0.09	8.08±0.19
	AST	42.2±1.07	43.4±0.68	42.4±0.87	40.2±0.58
	ALT	30.4±0.68	30.6±0.24	31.6±0.51	27.6±0.87*
	ALP	119.2±4.64	112.4±5.35	109.2±4.35	104±2.82*
	Creatinine	0.9±0.13	0.96±0.12	0.96±0.17	0.94±0.11
	Glucose	129±1.70	115.8±0.80*	118.4±1.21*	116.2±0.37*
	Urea	13.8±0.73	13.0±0.71	14.4±0.51	16.0±0.45*
	Cholesterol	52.8±4.28	55.8±5.81	67.2±4.87*	60.2±2.91

*Significant at * p<0.05 compared to control.*

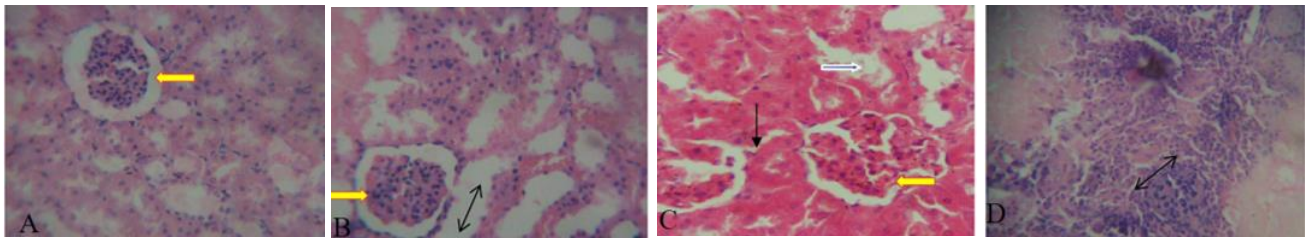


Plate 1:
The figures above show H&E staining of the kidney (X 100 magnification). **A**. Control group having normal nephron with normal glomerulus (yellow arrow). **B**. 50 ppm group showing normal glomerulus (yellow arrow) and mild sloughing off of nephron tubules (black arrow). **C.** 100 ppm group showing normal glomerulus (yellow arrow); some of the renal tubules are collapsed with diminishing lumen (black arrow) and sloughing off of nephron tubules. **D** 200 ppm group having nephritis due to severe infiltration of inflammatory (spanning arrow).

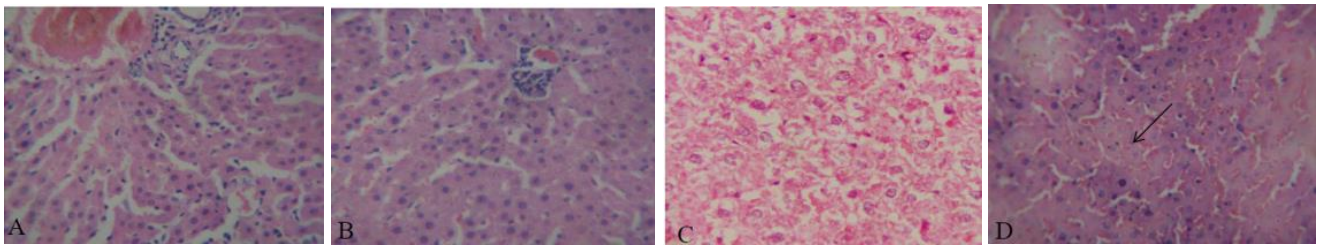


Plate 2:
The figures above show H&E staining of the Liver (X100 magnification). **A.** Control group having normal hepatic cells. **B.** 50ppm V group also having normal hepatic cells. **C.** 100ppm V group having normal appearance of sinusoids and **D.** 200ppm V group showing moderate congestion of blood vessels (black arrow).

DISCUSSION

There are conflicting reports on the effects of vanadium on body weight. Some experimental works have reported significant decrease in body weight of animals exposed to vanadium in comparison to control (Adachi *et al*., 2000; Olopade *et al*., 2011), while some others reported no significant difference in body weight (Dai *et al*., 1995; Scibior, 2005). Most reports on body weight loss noticed are either due to administration of high concentration of vanadium orally for a short duration or via inhalation and injection. However, in our experiment we observed that vanadium exposed group at 50ppm had significant increase in body weight beginning from the 6^{th} week to the 10^{th} week. The increase in body weight correspond with the work of Schroeder and Mitchener, 1975 where male mice given vanadium at 5mg/L as vanadyl sulfate in drinking water for life span had significantly higher body weights than control. Likewise, Krosniak *et al*., (2019) reported a significant increase in body weight in diabetes model of mice exposed to vanadium.

Vanadium in this research showed toxic effects in most of the haematological variables. As observed, vanadium toxicity was dose dependent with some significant toxic effect seen at 100 ppm and the most toxic effect recorded at 200ppm. Noteworthy decrease was detected in RBC count, Hb concentration, PCV, WBC count, and Lymphocyte, while a significant increase in Neutrophils was seen. The result corresponds to the work of Scibior *et al*., (2006) who reported that vanadium significantly decreases RBC count and Hb concentration at a vanadium concentration of 0.125mg V/mL administered in drinking water for 6 weeks. Also, Obianime *et al*., (2009) reported that ammonium metavanadate significantly decrease Hb, PCV, WBC and lymphocytes over a 28 days treatment. Their observation also implies that the effect of vanadium was dose and time dependent. However, the no significant difference observed in these variables at 50ppm and 100ppm correspond to the report of Dai *et al*., (1995) that administered 0 or 9.7mg vanadium/kg body weight for 12 weeks and no difference in haematological parameters was observed between the groups. Increase in neutrophil-lymphocyte ratio has been reported in various disease condition as a marker for inflammation (Salami *et al*., 2015) and this variable was markedly increased in the 200ppm compared to control. This is indicative that vanadium at high concentration is capable of causing systemic inflammation and eventually oxidative stress. The activation of neutrophil has been reported as one of the mechanisms by which vanadium mediate the formation of hydroxyl radical (OH^-). Thus, from our study as well as other reports, it shows that with increasing concentration, vanadium seems to affects haematological parameters.

Vanadium at 200ppm significantly reduces plasma levels of ALP and ALT and also shows a reduction in the level of AST which is a confirmation of the work of Adachi *et al.* 2000 who reported that vanadium showed a significant decrease in these parameters in rats fed with diet containing 100ppm of sodium metavanadate. It has also been reported that vanadium is a potent inhibitor of the enzyme alkaline phospatase (ALP) (Lopez *et al*., 1976), as well as other enzymes including phospho-transferase (Lindquist *et al*., 1973), Na^+ and K^+ ATPases (Cantley *et al*, 1978), Ca-Atpase (O'Neil *et al*., 1979). However, vanadium at 50ppm and 100ppm had no substantial effect in ALT and AST.

A decrease in glucose level was observed in all vanadium treated groups. This buttresses the anti-diabetic property of vanadium as vanadium has been reported to normalize glucose level in insulin-dependent diabetic rats and humans as well as inhibit glucose-6-phosphatase which is a key enzyme involved in the final step in gluconeogenesis and glycogenolysis (Cam *et al*., 2000; Kiersztan *et al*., 2004; Saima 2013). Urea concentration was seen to be increased in vanadium group treated with 100ppm and 200ppm sodium metavanadate and was significant at 200ppm. Also, comparing the cholesterol level, vanadium showed an increase in all vanadium treated group and was significant in group 3 (100ppmV). These corroborate the work of Mona *et al.*, (2007) where it was reported that cat fish fed with diet containing 15mg/kg vanadium causes a significant increase in urea and cholesterol levels.

The significant changes observed in serum biochemical variables is an indication of the toxic action on vanadium on the kidney and liver. Histological observations on the kidney revealed severe inflammation of the nephron and in the liver, there was blood congestion. The liver and kidney are major organs for vanadium toxic effect and have been reported to be major sites for vanadium bioaccumulation after absorption (Sabbioni *et al.*, 1978; Ramanadham *et al.*, 1991; Sanchez*et al*., 1998). Thus, these organs are severely affected irrespective of the mode of vanadium exposure however, since the liver is an accessory organ to the gastrointestinal tract, substances consumed first pass through the liver before entering the general circulation. This could be a probable reason why the toxic effect of vanadium in our study was more on the liver than the kidney and it is consistent with the report of Kemeir *et al*., (2011).

In conclusion, vanadium proved to be beneficial by enhancing body weight at low concentration, while evoking systemic inflammation at high concentrations. Vanadium also is indeed a potential agent for diabetes treatment as all concentrations of vanadium in this study caused a decrease in glucose level.

REFERENCES

Adachi A, Asai K, Koyama Y, Matsumoto Y and Okano T. (2000). Subacute vanadium toxicity in rats: Journal of Health Science, 46(6): 503-508.

Aragón, A.M. and Altamirano-Lozano, M. (2001). Sperm and testicular modifications induced by subchronic treatments with vanadium (IV) in CD-1 mice. Reproductive Toxicology 15: 145–151.

Barrio D.A., and Etcheverry S.B. (2006). Vanadium and bone development: putative signaling pathways. Can. J. Physiol. Pharmacol. 84:677-686.

Cam, M. C., Brownsey, R. W. and McNeill, J. H. (2000). Mechanisms of vanadium action: insulin-mimetic and insulin-enhancing agents. Canadian Journal of Physiology and Pharmacology, 78, 829–847.

Cantley, L.C., Jr, Josephson, L., Warner, R., Yanagisawa, M., Lechene, C. and Guidotti, G. (1977). Vanadate is a potent (Na,K)-ATPase inhibitor found in ATP derived from muscle. J. biol. Chem., 252, 7421–7423.

Dabros, W., Goc, A., Turyna, B., and Kordowiak, A.M. (2006). Sodium metavanadate affected control and streptozotocin-diabetic rat liver golgi complexes. Polish Journal of Pathology, 57(2):91-97. Abstract from PubMed 17019971.

Dacie, J.V. and Lewis, S.M. (1994). Practical Haematology 7thedition ELBS with Churchill Livingstone. Longman group UK. 5-82: 160-175.

Dai, S., Vera, E., McNeill J.H. (1995). Lack of haematological effect of oral vanadium treatment in rats. Pharmacology and Toxicology 76(4):263-268.

Das, S., Chatterjee, M., Janarthan, M., Ramachandran, H., and Chatterjee, M. (2012). Vanadium in Cancer Prevention, in Vanadium: Biochemical and Molecular Biological Approaches, Part IV: Medicinal Functions of Vanadium, ed. H. Michibata, Springer: London-New York, pp. 163–185.

Egborge .A.B.M. (1994). Water Pollution in Nigeria. Vol.1. Biodiversity and Chemistry of Warri River, Ben Miller Books, Warri, p.331.

Francik, M., Krosniak, M., Barlik, M., Kudla, A., Grybos, R., and Librowski, T. (2011). Impact of vanadium complexes treatment on the oxidative stress factors in Wistar rats' plasma. Research Article. Hindawi Publishing Corporation. Bioinorganic Chemistry and Applications. Article ID 206316, 8 pages.

Gornal, A.G., Bardawill, C.J, David, M.M. (1949). Determination of serum protein by means of Biuret reaction. Journal of Biological Chemistry.177:571-576.

Gummow, B. (2011). Vanadium-Environmental pollution and health effects. School of Veterinary and Biomedical Sciences, James Cook University, Townsville; QUB, Australia, Elsevier P.V. page 628-636

Hoff .J. (2000). Methods of blood collection in the mouse. Lab animal Technique. 29(10): 47-53.

IPCS (International Programme on Chemical Safety). 2001. Vanadium pentoxide and other inorganic vanadium compounds. Concise International Chemical Assessment Document 29. World Health Organization, Geneva, Switzerland.

Kemeir, M.E.A. (2013). The protective effect of vanadium sulphate on ethanol-induced gastric ulcer. Bahrain Medical Bulletin, Vol. 35 (4) 9 pp.

Kiersztan, A., Winiarska, K., Drozak, J. (2004). Differential effects of vanadium, tungsten and molybdenum on inhibition of glucose formation in renal tubules and hepatocytes of control and diabetic rabbits: beneficial action of melatonin and N-acet. Molecular and Cellular Biochemistry, 261 (1), 9–21.

Krosniak M, Szklarzewicz, J., Grybos, R., Tatar, B., Yildrim, M., Sahin, B., Yuksek N.D., and Ustundag, M. (2019). The influence of chronic supply of vanadium compounds on organ weights and body mass in animal diabetes model (NZO). Sci, Tech. Innov. 4(1): 63-73.

Lin T.S, Chang C.L and Shen F.M (2004). Whole blood vanadium in Taiwanese college students. Bull Environ Contam Toxicol 73: 781–786.

Lindquist, R. N., Lynn, J. L. Jr and Lienhard, G. E. (1973). Possible transition- state analogs for ribonuclease. The complexes of uridine with oxovanadium (IV) ion and vanadium (V) ion, Journal of the American Chemical Society, 95, 8762–8768.

Lopez, V., Stevens, T. and Lindquist, R. N. (1976). Archives of Biochemistry and Biophysics, 75, 31–38.

Marcano L, Carruyo I, Fernández Y, Montiel X, Torrealba Z (2006). Determination of vanadium accumulation in onion root cells (Allium cepa L.) and its correlation with toxicity. Biocell 30: 259–267.

Mona, S.Z, Nevin, E.S and Mostafa, H.O. (2007). Effect of Vanadium toxicity on Biochemical, Haematological and Clinicopathological changes in Clarias Lazara present in the River Nile. American- Eurasian J. Agric. and Environ. Sci, 2(6); 741-745. ISSN 1818-6769.

Nadal, M,, Schuhmacher M., Domingo J.L (2004): Metal pollution of soils and vegetation in an area with petrochemical industry. Sci Total Environ 321: 59–69.

Ngwa, H.A, Kanthasamy A, Anantharam, V, Song, C, Witte, T, Houk, R.S and Kanthasamy A.G. (2009). Vanadium induces dopaminergic neurotoxicity via protein kinase C-Delta dependent oxidative signaling mechanisms: Relevance to etiopathogenesis of parkinson's disease. Toxicol Appl pharmacol. 240(2): 273-285.

O'Neil, S.G., Rhoads, D.B., and Racker, E. (1979). Vanadate inhibition of sarcoplasmic reticulum Ca2+ ATO-ase and other ATP-ase. Biochemical and Biophysical research communications.; 89: 845-850.

ObianimeA, W., Gogo-Abite, M. and Roberts, I.I. (2009). The effects of Ammonium Metavanadate on Biochemical Hormonal, Haematological and Histopathological Parameters of the female wistar rats. Nigerian Journal of Physiological sciences 24 (2): 187 - 194

Olopade J.O, Fatola I.O and Olopade F.E (2011). Vertical administration of vanadium through lactation induces behavioural and neuromorphological changes: protective role of vitamin E. Niger. J. Physiol Sci. 26 (1): 55-60

Omayone, T.P., Salami, A.T., Oluwole, F.S. and Olaleye, S.B. (2016). Gastroprotective effect of vanadium in rats-roles of gastric acid and nitric oxide. J. Afr. Ass. Physiol. Sci.; 4(1): 32-40.

Połedniok, J. and Buhl, F. (2003): Speciation of vanadium in soil. Talanta 59: 1–8.

Pourang N., Nikouyan A., and Dennis, J.H. (2005). Trace element concentrations in fish, surficial sediments and water from northern part of the Persian Gulf. Environ Monit Assess 109: 293–316.

Ramanadham, S., Heyliger, C., Gresser, M., Tracey, A., and McNeill J (1991) The distribution and half-life for retention of vanadium in the organs of normal and diabetic rats orally fed vanadium (IV) and vanadium(V). Biological trace element research, 30:119–124.

Sabbioni, E., Marafante,. E, Amantini, L., Ubertalli, L, Birattari C (1978). Similarity in metabolic patterns of different chemical species of vanadium in the rat. Bioinorganic chemistry, 8:503–515.

Saima S. (2013). The potential effect of vanadium compounds on glucose-6-phophatase. Bioscience Horizon Volume 6: 1-11.

Salami A.T., Adeola B.O., Iyiola T.O., Omayone T.P., Oluwole F.S and Olaleye S.B. (2015). Antioxidative action of manganese treatment in delayed healing of acetic acid-induced ulceration in rat stomach. Journal of African Association of Physiological Sciences. 3 (2): 67-78.

Sanchez D, Colomina M, Domingo J (1998). Effects of vanadium on activity and learning in rats. Physiology and behaviour, 63(3):345–350.

Sanchez-Gonzalez, C., Moreno, L., Lopex-Chaves, C., Nebor, E., Pietschmann, P., Rodriguez-Nogales, A., Gelvez, J., Montes-Bayon, M., Sanz-Medel, A., Llopis, J. (2017). Effect of vanadium on calcium homostasis, osteopontin mRNA expression, and bone microarchitecture in STZ-induced diabetic rats. Metallomics. DOI:10.1039/C6MT00272B.

Scibior, A. (2005). Some selected blood parameters in rats exposed to vanadium and chromium via drinking water. Trace Element Electrolytes, 22(1):40-46. Abstract from EMBASE 2005083129

Wogu, M.D., and Okaka, C.E. (2011): Pollution studies on Nigerian rivers: heavy metals in surface water of Warri river, Delta State. Journal of Biodiversity and Environmental Sciences (JBES). Vol. 1. No. 3, p: 7-12.

Wogu, M.D., and Okaka, C.E. (2011). Pollution studies on Nigerian rivers: heavy metals in surface water of Warri river, Delta state. Journal of Biodiversity and Environmental Sciences (JBES). ISSN: 2220-6663. Vol. 1, No 3, p. 7-12

Worle-Knirsch, J.M., Kern, K., and Schleh, C. (2007). Nanoparticulate vanadium oxide potentiated vanadium toxicity in human lung cells. Environ. Sci. Technol., 41(1): 331-336.

Wright MT, Belitz K (2010) Factors controlling the regional distribution of vanadium in groundwater. Ground Water 48: 515–525.

Niger. J. Physiol. Sci. 35 (December 2020): 155 - 159
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Consumption of Calcium Carbide-Ripened Banana by Pregnant Rats May Programme for Infertility in Female Offspring

***Iyare C.O., Iyare E.E., Nwachukwu D.C. and Anyaehie U.B.**

Department of physiology, Faculty of Basic Medical Sciences, College of Medicine University of Nigeria Enugu campus, Enugu state, Nigeria

Summary: One of the substances used in force ripening fruits is commercial grade calcium carbide (CaC2) which contains impurities such as arsine and this has been associated with low birth weight and fetal loss. There is thus a need to further investigate additional risks on offspring. This study was thus designed to evaluate the possible effects of maternal consumption of banana pulp, force ripened with CaC_2, on the offspring. Sixteen pregnant rats were randomly divided into two test groups and controls of four rats each. Two test groups were fed with pelletized feed mixed with banana pulp ripened by commercial grade CaC_2 at concentrations of 50g/5kg and 100g/5kg while the controls had a group fed with pelletized feed mixed with normal ripened banana and another had only pelletized feed. This feeding pattern was done morning and evening *ad libitum* throughout the gestation period of twenty-one days after which only pelletized feed and water was administered. At delivery, all male offspring were separated and each dam was allowed eight female pups to nurse. Upon weaning after twenty-one days, the mothers were removed leaving eight female offspring in each group. Development of their reproductive system was monitored and recorded using parameters such as vaginal opening day (VOD) and reproductive hormonal assay at the sixth week. A fertility test was also carried out by introducing viable male rats for mating at sixth week postpartum. Trace amount of arsenic was found in the banana pulp of 100g/5kg CaC_2 group (0.35ppb). CaC_2 exposure was related to delayed onset in puberty, decreased serum FSH and a decreased fertility rate in the 100g/5kg CaC_2 group ($p<0.05$). Consumption of contaminated CaC_2 ripened fruits exposes humans to arsenic acid which has harmful effects on reproductive development of offspring.

Keywords*: calcium carbide, arsenic, reproductive hormone, Vaginal opening day, postnatal reproductive development, fertility rate*

**Address for correspondence: cordie2age@gmail.com; Tel: +234-8131232377*

Received - June 2020; Accepted- October 2020

INTRODUCTION

Environmental contaminants including endocrine disrupting chemicals are causing adverse reproductive health worldwide (Rashtian *et al*. 2019). The intrauterine and early childhood periods are the most vulnerable windows for chemicals that may impair growth and organ development (Grandjean *et al*., 2008). Even the least concentration of these common environmental pollutants that would appear harmless in the short term to an adult will be very harmful to the developing fetus and during early child development (WHO 2002). Consumption of good and ripe fruits is a healthy diet habit recommended for all and that place an increase demand on the supply in Nigeria. There is thus the propensity to initiate artificial ripening of these fruits. Chemicals are commonly employed as artificial ripening agents and the substance commonly used in Nigeria is calcium carbide because it is relatively cheap (Singal, Kumud, and Thakral, 2012). Calcium carbide (CaC_2) in its pure form is not toxic but commercial grade CaC_2 also known as *masala* is extremely hazardous to the human body as it contains traces of arsenic and phosphorus. It is banned in many countries of the world, but it is freely available in Nigeria, India, Pakistan and other countries (Dhua and Siddiqui, 2010). Several studies suggest association between arsenic exposure and adverse pregnancy outcomes such as spontaneous abortion, stillbirth, and infant death (Ahmad *et al*. 2001; Milton *et al*. 2005; von Ehrenstein 2006; Rahman *et al*. 2007). However, limited studies exist on the programming of intra-uterine exposure to impure commercial grade CaC_2 on fertility of the surviving offspring and the molecular mechanism underlying this deleterious effect.

MATERIALS AND METHODS

Experimental layout for ripening of banana: Unripe matured bananas (*Musa spp*) was purchased locally and authenticated in the department of Plant Science and Biotechnology, University of Nigeria, Nsukka and was assigned a voucher's number UNH No 813. The banana was ripened using commercial grade calcium carbide in the form of solution according to the method used by Chandel et al. (2018). For the control groups, control one (T0) had rats fed with pelletized feed and water only while control two (T1) had five-kilogram banana fruit placed in five-kilogram capacity carton and allowed to ripen naturally (without using CaC_2) at ambient temperature. For the test groups, five-kilogram banana fruit was dipped in five litres of water containing 50g of CaC_2. This served as T2. Another five-kilogram banana fruit was dipped in five litres of water containing 100g of CaC_2. This was T3. T2 and T3 were kept

for 30 minutes after which the fruits were removed from the solution and air dried to remove adhering moisture. The treated fruits were placed in five-kilogram capacity carton each and allowed to ripen at ambient temperature. After which, banana from each group was separately fed into a Qlink Blender model QBL-20L330 China, and then homogenized. The resulting puree (juice) was introduced into plastic bottles, properly labelled and preserved in a refrigerator at 15ºC which was later used in the animal experiment according to Gbakon *et al* (2018)**.** Although the doses of CaC_2 used in this study have been used by a previous study (Chandel *et al*., 2018), the rationale behind the adoption of the doses was to ascertain the possible health effect that can arise from a concentration this low.

Experimental design: Sixteen nulliparous (weighing between 170-220g) rats were used for this study. After successful mating and pregnancy was confirmed by the presence of vaginal plug, the rats were randomly assigned into four groups namely T0, T1, T2, and T3. Each group had four rats each. The rats were fed with pelletized mash. Pulp from the previously ripened banana fruits from each group was taken for estimation of arsenic residues in the banana from the peel and pulp separately for each treatment by using wet digestion according to the method of Chandel *et al*. (2018), using ICP-AES (Inductively Coupled Argon Plasma-Atomic Emission Spectrometry, Buck Scientific 210 Variable Giant Pulse model). The level of arsenic residues in the various treatments was compared with the maximum contamination level of arsenic residue in fruits which is 0.05ppb (USDA, 2006).

The banana pulp was mixed with the pelletized mash and the rats in each group fed *ad libitum* according to Gbakon *et al* (2018). The blended banana was mixed with the rat feed according to the method by Igbinaduwa *et al*. (2016). The allotment of diet is shown in the table below.

Table 1:
Allotment of diet across the groups

Groups	CaC_2 level (g/kg of fruit)	Treatment diet
T0	0g	Normal rat feed +water
T1	0g	Banana pulp (without CaC_2) + feed + water
T2	50g/5kg	Banana pulp (with CaC_2) + feed + water
T3	100g/5kg	Banana pulp (with CaC_2) + feed + water

After delivery, the male pups were removed leaving only the female pups. Each dam was allowed eight female pups to nurse throughout the lactation period to eliminate any form of over nutrition or mal nutrition. At postnatal day twenty-one, the pups were weaned off while the mothers were removed from the groups. The vaginal opening day (VOD) for each rat per group was recorded and taken as the onset of puberty (Ojeda and Skinner, 2006). At Postnatal day 35 (fifth week postpartum), the week the vagina of rats opened in the control group, blood was taken from the dorsal aorta of four rats in the control group and subsequent groups at their respective vaginal opening day (VOD) for assay of reproductive hormone namely follicle stimulating hormone (FSH), Luteinizing hormone (LH) and Estrogen (E_2). The hormones were estimated by radioimmunoassay (RIA). These rats were marked thereafter. The marked four pups in each group were later sacrificed after blood had been obtained from them by cervical dislocation and ovaries harvested and preserved in formalin for tissue processing while the remaining four were subjected to fertility test by introducing viable and mature males to the female pups. Confirmation of pregnancy was a sign of fertility. The fertility rate per group was calculated according to (Anjum and Reddy 2015) with slight modification as:

$$\frac{number\ of\ pregnant\ rats}{total\ number\ of\ mated\ rats} X\ 100$$

Statistical analysis: Findings were tabulated and analyzed with results expressed as mean ± SEM. Statistical analysis was done using one-way Analysis of Variance (ANOVA). The results were compared using Post-hoc (tukey) test. Results were considered significant at $p< 0.05$

Ethical consideration: All procedures used in this study adhere to the ARRIVE (Animals in Research: Reporting *in Vivo* Experiments) guidelines for reporting animal research (Kilkenny *et al*. 2010; Tilson and Schroeder, 2013) and the ethical standards of this experiment is in accordance with the guidelines provided by the CPCSEA and World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research involving experimental animals. It was equally approved by College of Medicine Research and Ethics Committee, University of Nigeria.

RESULTS

Arsenic residue analysis of CaC_2 ripened banana by atomic absorption: Between the treatment groups, significant elevation in mean arsenic deposition was observed in the peel of 50g/5kg CaC_2 and 100g/5kg CaC_2 treatments group ($p<0.05$) compared to naturally ripened banana group. Non-significant increase was observed in the pulp of the treatment groups in dose-dependent manner as seen in the Table 2.

Table 2:
Arsenic residue analysis of CaC_2 ripened banana by atomic absorption

Ripening treatments (T)	Arsenic residue (ppb) in different fruits parts	
	Banana Peel	Banana Pulp
T1 (naturally ripened)	0.00 ± 0.00	0.00 ± 0.00
T2 (dipping in 50g/5litres of CaC_2)	0.61 ± 0.19	0.30 ± 0.12
T3 (dipping in 100g/5litres of CaC_2)	1.28 ± 0.00*	0.35 ± 0.17

Values are mean ± SE
**significantly different from T2 at p<0.05*
Arsenic residue in the calcium carbide granules was found to be 1.71ppb.

Vaginal opening day (VOD) of female offspring exposed to prenatal CaC_2 ripened banana: In the Table 3, a Significant increase (P<0.05) was observed in vaginal opening day in 100g/5kg CaC_2 group when compared to control. There was a non-significant increase in the VOD of the naturally ripened banana group (T1) when compared with the control. A significant dose-dependent increase was also observed within the CaC_2 treatment group (T2 and T3).

Hormonal analysis by radioimmunoassay (RIA) on reproductive hormone of female offspring exposed to prenatal CaC_2 ripened banana: Significant dose-dependent decrease (P<0.05) was observed in mean plasma levels of FSH with all doses of CaC_2 compared to control. Within the treatments groups, 50g/5kg CaC_2 group showed a non-significant (p<0.05) increase in plasma levels of FSH, LH and estrogen compared to 100g/5kg CaC_2 group. The naturally ripened banana group (T1) significantly increased the levels of FSH, LH and estrogen (p<0.05) when compared to the CaC_2 treatment groups as shown in Table 4.

Fertility rate of female offspring exposed to prenatal CaC_2 ripened banana: A significant dose-dependent decrease in fertility rate was observed in 100g/5kg CaC_2 group when compared with the control and naturally ripened banana groups. A non-significant decrease in fertility rate was observed within the treatment groups as seen in the Table 5.

Table 3:
Vagina opening day (VOD) of female offspring exposed to prenatal CaC_2 ripened banana (*mean ± SE, n= 8)*

GROUPS	Vagina opening day (days)
TO	35 ± 0.74
TI	33 ± 0.62†
T2	36 ± 0.16*
T3	44 ± 0.48‡

* *Significantly different from T3 at p < 0.05*
† *Significantly different from T3 at p < 0.05*
‡ *Significantly different from T0 at p < 0.05*

Table 4:
Hormonal analysis by radioimmunoassay (RIA) on reproductive hormone of female offspring exposed to prenatal CaC_2 ripened banana (*mean ± SE, n= 4)*

Groups	FSH (mIU/mL)	LH (mIU/mL)	Estrogen (pg/mL)
T0	47.1±1.1	12.8±4.0	57.7±11.8
T1	44.0±1.6	20.6±0.6	67.8±4.6
T2	32.8±2.2*†	9.4±1.2†	37.9±3.7*†
T3	34.2±1.4*	9.6±1.1†	28.5±1.0*†

**significantly different from the control (T0) at p<0.05*
† *Significantly different from T1 at p<0.05*

Table 5:
Fertility rate of female offspring exposed to prenatal CaC_2 ripened banana

Groups	Fertility Rate (%)
T0	100
T1	100
T2	75
T3	50*

Values are expressed as mean ± SE, n=4;
**=significant when compared with T0 and T1 at p<0.05*

Histological observation of ovaries of female offspring exposed to prenatal CaC_2 ripened banana: From Plate 1, the graafian follicles of the naturally ripened banana group were very large with increased amount of follicular fluid when compared to other groups (Plate 1B). Among the treated group, the 50g/5kg group had same number of developing follicles but smaller with little or no fluid within the follicles while the 100g/5kg group had markedly reduced number of follicles with scanty follicular fluid (Plate1C & D). The number of developing follicles was observed to be more in the control group (Plate 1A) and it appeared to decrease with increase in the dose of CaC_2.

In Plate 2, the space between the granulosa cells and the theca interna is observed to be well demarcated in both control and naturally ripened banana groups while it is indistinguishable in both treatment groups as there is no marked demarcation between them. In Plate 2, the follicles are seen to be closed to each other in both the control and naturally ripened banana groups while they are markedly spaced in the treatment group and this increase is dose dependent.

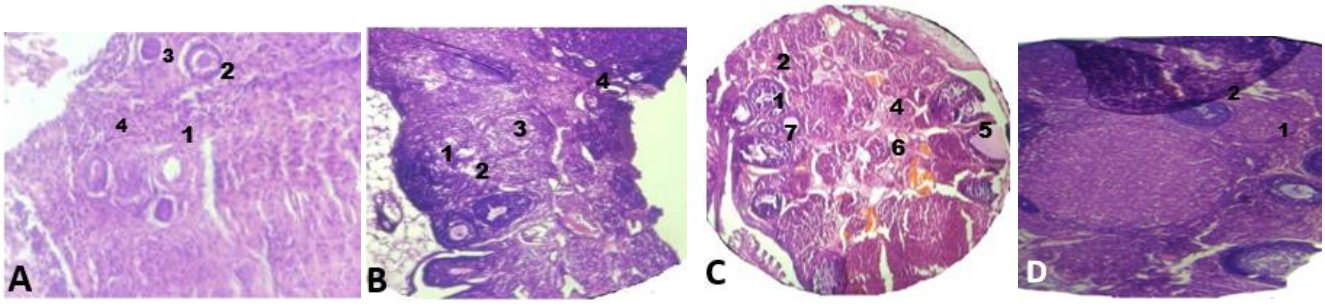


Plate 1:
Photomicrograph of ovary to offspring of control and CaC_2 treated mothers at puberty, showing number of developing follicles.(A) control (T0) showing normal proliferating developing ovarian tissue with the formation of primary follicles (B) Naturally ripened group (T1) showing normal proliferating developing ovarian tissue with the formation of primary follicles. (C) 50g/5kg CaC_2 group (T2) showing malformation of ovarian follicles with disappearance of the fluid in some follicles leading to solidification of some of the follicles. Hemorrhage can also be seen in the vascular channels. (D) 100g/5kg CaC_2 group (T3) showing reduction in number of developing ovarian follicles.

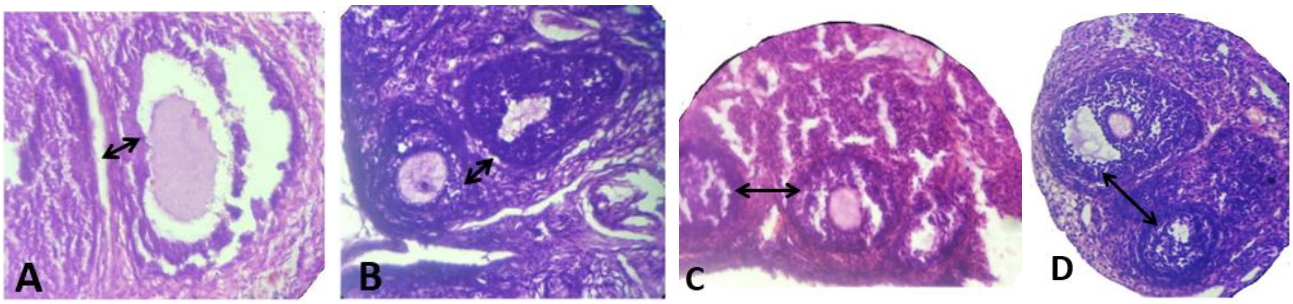


Plate 2:
Photomicrograph of ovaries to offspring of control and CaC_2 treated mothers at puberty, showing graafian follicle. The black arrows indicate the distance between the graafian follicles in each ovary.

The connective tissue around the follicles is moderately dense with clear vascular channels in the control and naturally ripened banana groups while it is relatively loose with congested vascular channels in the low dose group. However, the connective tissues in the high dose group are thicker and deeply hyalinized with fewer follicles Plate 2).

DISCUSSION

In this study we reported the effects of maternal exposure to CaC_2-ripened fruit on the reproductive development of the pups to unravel the mechanisms behind reproductive failures associated with arsenic exposure. It is evident from the result that maximum level of arsenic was found in the banana peel of 100g/5kg CaC_2 group but no significant difference in the concentration of arsenic was found in the banana pulp of both treatment groups. A possible explanation for this could be that the banana peel may act as a barrier preventing the influx of harmful chemicals to the pulp. A study has reported that some components of banana peel, such as Hydroxyl and Carboxyl of Pectin, have the ability to absorb heavy metals (Palacios, 2005). This could explain why the concentration of arsenic in the banana pulp, in both treatment groups, was fairly same despite the varied amount of CaC_2 used. All concentration of arsenic residue in the treatment groups exceeded the maximum contamination level of arsenic in fruits (0.05ppb) (USDA, 2006).

Age of vaginal opening and levels of circulating reproductive hormone have been used as predictors of puberty in mice (Kimberly *et al*., 1997). The observed delay in vaginal opening in group T3 (100g/5kg CaC_2 treated banana) could be linked to the aforementioned alterations in serum reproductive hormone. It has equally been reported that arsenic poisoning in trace amount can lead to one month's delay in the onset of puberty in female rats (Dávila-Esqueda *et al*., 2012). The mechanism explains that pre-pubertal exposure to arsine (III) acts peripherally to suppress circulating levels of IGF-1 resulting in delayed sexual maturation. It went further to identify a critical window of increased susceptibility to arsine (III) that may have a lasting impact on female reproductive function (Reilly *et al*., 2014). Contrary to the above, a study has reported that carbide accelerates puberty onset (Bafor *et al*., 2019).

Levels of circulating LH have been used as predictors of puberty in mice (Risma *et al*. 1997). Elevated serum LH, which is positively linked to precocious puberty (Risma *et al*. 1997), was observed only in the group that ate naturally ripened banana. A possible explanation for the observed increase in stimulation of LH and estrogen secretion could be banana-induced as certain meals have been reported to induce stimulation of hormone secretion (Schreihofer *et al*., 1996). Inorganic arsenic has been reported to suppress ovarian steroidogenesis, prolongs diestrus, and degenerates ovarian follicular cells (Navarro *et al*., 2004; Chattopadhyay *et al*., 2001; Zhang *et al*., 2000), which explains the observed decrease in serum concentration of LH, FSH and estrogen in this study. The mechanism behind the observed arsenic toxicity in the female reproductive system could be arsenic-induced changes in the levels of catecholamines in the brain. The elevation in serotonin and decrease in norepinephrine in the midbrain and diencephalon could lower gonadotrophin synthesis and secretion. Low gonadotrophin levels could in turn decrease activities of ovarian regulatory enzymes for steroidogenesis, a reasonable explanation for the reduction in all three hormones. The observed low FSH level may contribute to the observed decreased number of healthy follicles and increased number of malformed follicles seen in the treatment groups. Studies have equally shown that Arsenic also causes toxicity to estrogen production by interfering with its signaling pathways (Chatterjee and Chatterji, 2010; Bae-Jump *et al*., 2008; Watson and Yager, 2007). Watson and Yager (2007) showed that Arsenic disrupts the estrogen signalling pathways by suppressing the action of estradiol on the uterus and interaction of estrogen receptors with some transcription factors.

A significant decrease in fertility rate was seen in the 100g/5kg CaC_2 group when compared to the control ($p<0.05$). Bafor *et al*. (2019) reported that fruits ripened with calcium carbide negatively alter the female reproductive physiology. The notable difference in fertility between the control and treatment groups under experimental conditions, were due to the alterations in levels of reproductive hormones as well as changes in the architecture of the follicles.

A limitation to this study is the fact that attempt to get pure form of calcium carbide for this study proved abortive. Further work will be done with the pure form of calcium carbide once available to ascertain if the chemical in its pure form has any negative effect in the body.

In conclusion, consumption of commercial grade CaC_2 ripened fruits during pregnancy exposes humans to a significant deleterious effect on puberty onset, and fertility rate of female offspring.

REFERENCES

Ahmad, S.A., Sayed, M.H. and Barua, S. (2001). Arsenic in drinking water and pregnancy outcomes. Environ Health Perspect. 109: 629 – 631.

Anjum, M.R. and Reddy, P.S. (2015). Recovery of lead-induced suppressed reproduction in male rats by testosterone. Andrologia, 47(5):560-567.

Bae-Jump, V.L., Zhou, C., Boggess, J. F., and Gehrig, P. A. (2008). Arsenic trioxide (As2O3) inhibits expression of estrogen receptor—alpha through regulation of the mitogen-activated protein kinase (MAPK) pathway in endometrial cancer cells. Reproductive Sciences, 15:1011-1017.

Bafor, E.E., Greg-Egor, E., Omoruyi, O., Ochoyama, E. and Omogiade, G.U. (2019). Disruptions in the female reproductive system on consumption of calcium carbide ripened fruit in mouse models. Heliyon, 5(9)e02397.

Chandel, R., Sharma, P.C. and Gupta, A. (2018). Method for detection and removal of arsenic residues in calcium carbide ripened mangoes. Journal of Food Processing and Preservation, 42(2): e13420.

Chatterjee, A., and Chatterji, U. (2010). Arsenic abrogates the estrogen-signaling pathway in the rat uterus. Reprod. Biol. Endocrinol. 8:80

Chattopadhyay, S., Ghosh, S., Debnath, J., and Ghosh, D., (2001). Protection of sodium arsenite-induced ovarian toxicity by coadministration of L-ascorbate (vitamin C) in maturewistar strain rat. Arch. Environ. Contam. Toxicol. 41, 83–89.

Dávila-Esqueda, M.E., Jimenez-Capdeville, M.E., Delgado, J.M., De la Cruz, E., Aradillas-Garcia, C. and Jimenez-Suarez, V. (2012). Effects of arsenic exposure during the pre- and postnatal development on the puberty of female offspring. Experimental and Toxicologic Pathology, 6:25–30.

Dhua, R. and Siddiqui, M.W. (2010). Eating artificially ripened fruits is harmful. Current science, 99:1664 – 1668.

Gbakon, S.A., Ubwa, T.S., Ahile, U.J., Obochi, O.G., Nwannadi, I.A., and Yusufu, M.I. (2018). Studies on Changes in Some Haematological and Plasma Biochemical Parameters in Wistar Rats Fed on Diets Containing Calcium Carbide Ripened Mango Fruits. International Journal of Food Science and Nutrition Engineering, 8(2):27-36.

Grandjean, P., Bellinger, D., Bergman, A., Cordier, S., Davey-Smith, G., and Eskenazi, B. (2008). The faroes statement: human health effects of developmental exposure to chemicals in our environment. Basic Clin Pharmacol Toxicol, 102: 73–75.

Igbinaduwa, P. and Aikpitanyi, R. (2016). Calcium carbide induced alterations of some haematological and serum biochemical parameters of wistar rats. Asian Journal of Pharmaceutical and Health Sciences, 6:1396-1400.

Kilkenny, C., Browne, W., Cuthill, I., Emerson, M. and Altman, D. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biology, 8(6), e1000412.

Kimberly, A., Risma, A.N. and Hirshfield, J.H. (1997). Elevated Luteinizing Hormone in Prepubertal Transgenic Mice Causes Hyperandrogenemia, Precocious Puberty, and Substantial Ovarian Pathology. Endocrinology, 138:3540–3547.

Milton, A.H., Smith, W., Rahman, B., Hasan, Z., Kulsum, U. and Dear, K. (2005). Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. Epidemiology, 16: 82–86.

Navarro, P.A., Liu, L., and Keefe, D.L. (2004). In vivo effects of arsenite on meiosis, preimplantation development, and apoptosis in the mouse. Biology of reproduction, 70: 980-985.

Ojeda, S.R. and Skinner, M.K. (2006). Puberty in the Rat. Knobil and Neill's Physiology of Reproduction, 2061–2126.

Palacios, A. (2005). Use of Banana Peel Based Filters to Reduce Heavy Metals in Wastewater. SPE Latin American and Caribbean Petroleum Engineering Conference. doi:10.2118/177250-ms

Rahman, A., Vahter, M., Ekström, E.C., Rahman, M., Mustafa, A.H.M. and Wahed, M.A. (2007). Association of arsenic exposure during pregnancy with fetal loss and infant death: A cohort study in Bangladesh. American Journal of Epidemiology, 165: 1389–1396.

Rashtian, J., Chavkin, D.E. and Merhi, Z. (2019). Water and soil pollution as determinant of water and food quality/contamination and its impact on female fertility. Reproductive Biology and Endocrinology, 17:5.

Reilly, M.P., Saca, J.C., Hamilton, A., Solano, R.F., Rivera, J.R. and Whitehouse-Innis, W. (2014). Prepubertal exposure to arsenic (III) suppresses circulating insulin-like growth factor-1 (IGF-1) delaying sexual maturation in female rats. Reproductive Toxicology, 44:41–49.

Risma, K. A., Hirshfield, A. N., and Nilson, J. H. (1997). Elevated luteinizing hormone in prepubertal transgenic mice causes hyperandrogenemia, precocious puberty, and substantial ovarian pathology. Endocrinology, 138:3540-3547.

Schreihofer, D. A., Renda, F., and Cameron, J. L. (1996). Feeding-induced stimulation of luteinizing hormone secretion in male rhesus monkeys is not dependent on a rise in blood glucose concentration. Endocrinology, 137: 3770-3776.

Singal, S., Kumud, M. and Thakral, S. (2012). Application of apple as ripening agent for banana. Indian Journal of Natural Products and Resources, 3:61–64.

Tilson, H.A. and Schroeder, J.C. (2013). Reporting of Results from Animal Studies. Enviromental Health Perspectives, 121: 320–321.

United States Department of Agriculture (USDA), (2006). Foreign Agricultural Services GAIN Report. Global Report No. CH6064, Chinese People's Republic of FAIRS products. Specific Maximum Levels of Contaminants in Foods, Jim Butterworth and Wu Bugang, 1-60.

von Ehrenstein, O.S., Guha Mazumder, D.N., Hira-Smith, M., and Ghosh, N. (2006). Pregnancy Outcomes, Infant Mortality, and Arsenic in Drinking Water in West. American Journal of Epidemiology, 163: 662–669.

Watson, W.H., and Yager, J. D. (2007). Arsenic: extension of its endocrine disruption potential to interference with estrogen receptor-mediated signaling. Toxicological sciences, 98: 1-4.

World Health Organisation, (2002). Children's health and environment : a review of evidence : a joint report from the European Environment Agency and the WHO Regional Office for Europe /cedited by Giorgio Tamburlini, Ondine S. von Ehrenstein and Roberto Bertollini.

World Health Organisation, (2011). Safety evaluation of certain contaminants in food. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/44520/9789241660631_eng.pdf

Zhang, C., Ling, B., Liu, J., and Wang, G. (2000). Toxic effect of fluoride-arsenic on the reproduction and development of rats. Wei sheng yan jiu= Journal of hygiene research, 29: 138-140.

Niger. J. Physiol. Sci. 35 (December 2020): 161 - 165
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Influence of Varying Degree of Wood Dust Exposure on Pulmonary Function and Respiratory Symptoms among Wood Workers in Kano, North Western Nigeria

***Nafisa Y. W[1]., Dawud, F. A[2]., Sulaiman, I[1]., Salisu A. I[1]., and Tukur M.A**

[1]*Department of Human Physiology, Faculty of Basic Medical Sciences, Bayero University, Kano. Kano, Nigeria*
[2]*Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.*
[3]*Department of Human Physiology, College of Medicine, University of Maiduguri. Maiduguri, Nigeria*

Summary: One of the major occupation-related health challenges encountered by wood workers is respiratory disorder, which usually results from breathing in noxious or toxic chemicals such as wood dust. The aim of this study is to evaluate the respiratory functions and symptoms among wood workers exposed to varying degrees of wood dust in Kano, Nigeria. This descriptive cross-sectional study was carried out among 370 randomly selected wood workers in Kano wood market. Lung function test was performed, while semi-structured interviewer administered questionnaire was used to rate respiratory symptoms. The study demonstrated that there was low percentage predicted force expiratory volume at one minute (PPFEV1) and percentage predicted ratio of FEV1 and FVC, whereas, the percentage predicted forced vital capacity (PPFVC) of the respondents across all age groups remained unchanged. Similarly, a negative correlation was observed between degree of exposure to the hazards and lung function of the workers (r = -0.655, P-Value = 0.0001). A statistically significant association existed between exposure to wood dust and respiratory symptoms, thereby contributing to the observed manifestation of respiratory symptoms such as chronic cough, corrhiza, breathlessness and wheezing among 61% of wood dust exposed workers.

Keywords*: Wood workers, Wood dust, Occupational hazard, Lung function, Respiratory symptoms*

**Address for correspondence: anafisaywali@yahoo.com; Tel: +234-8037005079*

Manuscript received- September, 2019; Accepted - August 2020

INTRODUCTION

Occupational exposure to widely used chemical and biological substances in work environment has long been acknowledged as cause of numerous diseases (Kim *et al* ., 2011; Montano, 2014). Wood dust is one substance that is strongly associated with respiratory diseases and lung function impairment (Ratnasingam *et al* ., 2014; Masoud *et al* ., 2018). Wood Processing results in the formation of wood chips and dust which could be partly suspended in the air and inhaled by the workers (Anders *et al* , 2002). High moisture content in fresh wood makes it less airborne relative to dry wood, which produces more dust during processing such as sawing and sanding. Soft wood particles are more fibrous and usually larger and as a result are also less capable of becoming airborne (Workers Health Center Fact Sheet, 2004). Concentration of wood dust in the industry varies in depending on the type of woodwork. The highest exposures have generally been reported in wood furniture and cabinet manufacture, especially during machine sanding and similar occupations (with wood dust level frequently above 5mg/m3) (Wood Dust Study Group 1, 1995). And 5 mg/m3 inhalable exposure limit was adopted in 1988. However, reduced lung function impairment was observed after long-term exposure to dust from soft wood at levels of 1.3 mg/m3 (Hessel, 1995) and 3.8 mg/m3 (Shamssain, 1992). Wood dust generated in the processing of wood for a wide range of uses is a complex substance. It is composed of cellulose fibres, resins and contaminants such as fungal spores and other microbes, wood preservatives, coatings, sealants and glues (e.g formaldehyde, pentachlorophenol, glycols, copper, naphthanate, e.t.c). This mixture makes it difficult to determine a specific irritant or allergen (SAFE work, 2005). However, the irritation symptoms in wood dust exposure are generally caused by the physical nature of wood dust particle. Thus, particle size is an important factor. The size of the dust particle produced the amount of dust and resultant exposure to staff working in these areas depends on a number of factors including the equipment being used, the ventilation and extraction system in place, the state and type of timber, general ventilation in the area and use of personal protective equipment.

The smaller the dust particle the further it will travel into the lungs causing symptoms of inflammation or allergy after repeated exposure (SAFE Work, 2005). Despite the numerous information regarding the effect of wood dust on lung function and respiratory symptoms, there is paucity of studies on influence of varying degree of wood dust exposure on lung function and respiratory symptoms of wood workers. Therefore, the present investigation is to study the degree of exposure to dust and other hazards in wood industry and resultant lung function impairment and respiratory symptoms.

MATERIALS AND METHODS

Study design and location: The study is a descriptive cross–sectional, conducted in Na'ibawa wood market Kano, Nigeria. The source of raw materials for the market is largely from the southern part of Nigeria and some parts of north central geopolitical zone of the country. Some of the common woods used in the market include black Akpara, Congo Akpara, white Akpara, Mansonia, Mahogany and Iroko.

Participants: All wood workers in Na'ibawa market were part of the study. However, workers with history suggestive of chest infection, known cases of asthma and chronic obstructive lung disease (COPD) before starting the job, known hypertensive patients were excluded from the study.

Data collection: Data was collected using a semi-structured interviewer administered questionnaire. A Vitalograph Electronic Spirometer Compact II was used for lung function study, Bathroom scale (Model Hamason, China) for measuring weight in Kg and Stadiometer for measuring height in cm.

Exposure to wood dust was calculated using exposure-rating system adapted from a past study by Ministry of manpower Singapore in 2006. An exposure rating scale of 1 to 5 points, where 1 represent very low exposure and 5 represents very high exposure, while 3 equals medium exposure was used in the analysis (Ministry of manpower Singapore, 2006) (Table 1).

Lung function was assessed by comparing measured values with predicted reference values of lung function parameters (FVC, FEV_1, and FEV_1/FVC) for that age, weight and height automatically generated by the Vitalograph Compact II electronic Spirometer, based on Kundson's formula for calculating lung function parameters in blacks. A value of the measured parameters > 75% of its predicted value was adjudged as normal. A value of > 65% but less than 75% was interpreted as mild lung function impairment (restrictive or obstructive) while that > 50% but less than 65% was interpreted as moderate lung impairment, and any value less than 50% of its predicted was adjudged as severe impairment of lung function.

The relationship between degree of exposure to hazards of wood work and lung function was determined using correlation plot, and the strength of the association was obtained using correlation coefficient (r). Chi-square test (χ^2) was used to determine significant association between degree of exposure to hazards of wood work with lung function impairment and respiratory symptoms. A confidence interval of 95% used and a p-value of < 0.05 were considered significant.

Data analysis: Data collected were collated and analyzed using the statistical package for social science (SPSS) version 22 (SPSS Statistics for Windows, version 22. Armonk, NY: IBM Corp.). Quantitative variables were summarized using mean and standard deviation, while categorical variable were summarized using frequencies and percentages as appropriate.

Ethical consideration: Ethical clearance (protocol no: ABUTH/PGO/COMM/9 dated 13th September, 2006) and permission for the study were sought and obtained from the Medical Ethical Committee of Ahmadu Bello University, Zaria, and Kumbotso Local Government Kano, Kano State, respectively. Informed consent was obtained from each participant before enrolling for the study.

Table 1:
Exposure Rating modified from 'Method to Assess Occupational Exposure to Harmful chemicals.

Exposure Factor	Exposure index				
	1	**2**	**3**	**4**	**5**
Particle Size	Coarse, bulk wet material	Coarse and dry material	Dry and small partial size	Dry and fine material	Dry fine powdered material
Vapour	-	-	-	-	Vaporizing chemical
Hazard Control Measure	Adequate control with regular maintenance	Adequate control with irregular maintenance	Adequate control without maintenance (moderate/dusty)	Inadequate control, dusty	No control at all, very dusty
Amount Used Per Week	Almost negligible <1kg or	Little amount used 1 - <10kg	Medium amount, workers are trained on handling dust 10 - <100kg	Large amount used, workers trained 100 – 1000kg	Large amount used, workers not trained >1000kg
Duration Of Work Per Week	< 8 hours	8 – 16 hours	16 – 24 hours	24 – 32 hours	>32 hours

Calculation

$ER = [EI_1 \times EI_2 \times \ldots\ldots \times EI_n]^{1/n}$

Where;

EI = Exposure Index

n = number of factors used. *ER score = 1 – 5*

1 = Very low *2 = low* *3 = medium* *4 = High* *5 = very high*

RESULTS

Three hundred and seventy (370) wood workers were recruited for the study. The respondents' engaged in different types of wood work namely trading, sawing, sanding, joinery, staking and spraying. Majority of the wood workers, 99 (26.7%) were involved in sawing of wood, whereas those involved in spraying of the wood constitute only 42 (11.3%) of the 370 wood workers. The means and range of respondents' ages, weights and heights by the type of work they do are as shown in table 2.

Table 3 shows the mean ventilatory indices of the respondents by their age group. The result observed low percentage predicted force expiratory volume at one minute ($PPFEV_1$) across the ages 25-34 and 45-59 years. However, the percentage predicted forced vital capacity (PPFVC) of the respondents across all age group was normal. The percentage predicted ratio of FEV1/FVC across the age group 25 to 34 years was lower than normal. Table 4 observed that cough to be the most common symptom among the respondents 134 (36.0%) followed by sneezing 93 (25.0%). On the other hand, wheeze was the least common symptom among the wood workers 23 (6.0%). Overall, 228 (61.6%) of the wood workers had respiratory symptoms.

The degree of exposure of the wood workers to hazards in their places of work measured by exposure rating is depicted in figure 1. More than a quarter 116 (31.0%) of the exposed workers had high exposure rating (3-5). The degree of exposure to hazards in woodwork place was significantly associated with lung function ($\chi^2 = 261.1$, df = 1, p = 0.01) as shown in Table 5. Up to 113 (97.4%) of the woodworkers that had impaired lung function had high degree of exposure, whereas 229 (90.1%) of those that had normal lung function had low degree of exposure. Furthermore, a negative correlation was observed between degree of exposure to the hazards and lung function of the workers (r = -0.655, P-Value = 0.0001). As degree of exposure increases, lung function decreases as depicted in Figure 2. This study also observed that degree of exposure to hazards of the woodwork was significantly associated with prevalence of respiratory symptoms ($\chi^2 = 16.2$, df = 1, p = 0.001) as shown in Table 5

Table 2:
Mean Values of Age, Weight and Height of Respondents by Their Type of Work

Type of work (n)	Mean age in years (Range)	Mean weight in kg (Range)	Mean height in cm (Range)
Sawing (99)	33.9 (16-54)	66.1 (45-88)	168.8 (150-184)
Sanding (52)	36.5 (19-56)	64.4 (49-88)	166.9 (156-178)
Stacking (66)	32.5 19-56()	64.9 (48-92)	166.4 (150-178)
Joinery (50)	31.8 (17-56)	63.5 (41-90)	166.5 (155-178)
Spraying (42)	32.2 (18-55)	64.4 (48-84)	167.4 (154-181)
Trading (61)	29.1 (17-56)	63.6 (38-87)	168.2 (155-182)

Table 3:
Mean Ventilatory Indices of the Wood Workers by Their Age Group

Age group	FEV_1M(l) Mean ± S.D	FEV_1P(l) Mean ±S.D	$PPFEV_1$ (%) Mean ± S.D	FVCM(l) Mean ±S.D	FVCP (l) Mean ±S.D	PPFVC (%) Mean ± S.D	$PPFEV_1$ /PPFVC (%)Mean ±S.D
15-19	2.43± 0.50	3.05±0.10	79.59±14.89	2.97±0.44	3.50±0.40	85.33±9.92	77.86 ± 9.31
20-24	2.24± 0.51	3.14±0.28	75.80±15.26	3.11±0.42	3.52±0.34	88.72±10.46	75.81 ± 10.86
25-29	2.29± 0.30	3.10±0.26	*73.87±16.29	3.13±0.34	3.53±0.32	88.82±8.51	*74.16±10.46
30-34	2.40± 0.27	3.27±0.32	*73.60±15.28	3.16±0.42	3.79±0.41	83.53±7.72	*73.82 ± 8.14
35-39	2.54± 0.20	3.25±0.29	78.24±12.54	3.09±0.36	3.75±0.31	82.40±8.37	77.51 ± 10.29
40-44	2.53± 0.14	3.10±0.15	81.45±10.54	2.83±0.35	3.55±0.2	79.77±9.65	79.83 ± 7.76
45-49	2.22± 0.21	3.09±0.17	*72.18±15.58	2.82±0.32	3.45±0.13	81.87±9.72	75.86 ± 7.06
50-54	2.24± 0.20	3.11±0.15	*72.10±14.01	2.72±0.29	3.45±0.19	79.15±10.09	78.04 ± 5.87
55-59	2.07± 0.15	2.94±0.19	*70.94±14.34	2.70±0.19	3.24±0.17	83.45±5.40	78.28 ± 12.83

*Key:*Lung function impairment, FVCM = Measured FVC; FVCP = Predicted FVC; PPFVC = % predicted FVC; FEV_1M =Measured FEV_1; FEV_1P = Predicted FEV_1; $PPFEV_1$ = % predicted FEV_1; $PPFEV_1$ /PPFVC% = % predicted FEV_1 to % predicted FVC ratio.*

Table 4:
Common Respiratory Symptoms among the Respondents

Sneezing	93 (25.0)
Regular blocked nose	44 (11.8)
Runny nose	48 (12.9)
Wheeze	23 (6.0)
Breathlessness	58 (15.6)
Tightness of chest	76 (20.5)
Cough	134 (36.0)

DISCUSSION

Pulmonary function tests detect impairment in lung function and assess the effect of treatment or progress of a disease. Forced Expiratory Volume in one second (FEV_1), Forced Vital Capacity (FVC) and Peak Expiratory Flow Rate (PEFR) are simple routine procedures which can be undertaken by health workers without special training (Cromopton *et al* , 1999). FEV_1/FVC ratio is the forced expiratory volume in 1 second expressed as a percentage of forced vital capacity, and gives a clinical index of airflow.

Table 5:
Factors Associated with Degree of Exposure to Hazards of Wood Work

Factor	Degree of exposure		
	High Freq (%) (n=116)	**Low Freq (%) (n=254)**	**Chi-square (p- value)**
Lung function			
Normal (% predicted ratio ≥75%)	3 (2.6)	229 (90.2)	261.1 (0.0001)*
Impaired (% predicted ratio < 75%)	113 (97.4)	25 (9.8)	
Respiratory symptom			
Symptom	54 (46.6)	174 (68.5)	16.1 (0.0001)*
No symptom	62 (53.4)	80 (31.5)	

* denotes p<0.05, statistically significant association

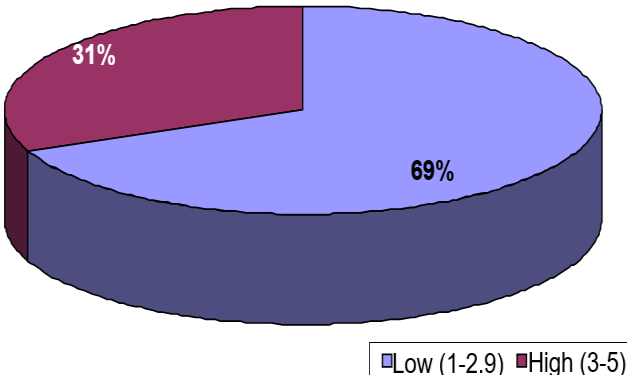


Figure 1:
Exposure Rating (ER) of Wood Workers

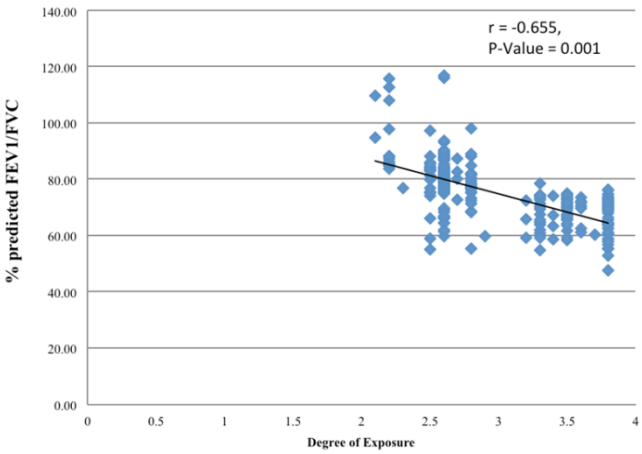


Figure 2.
Relationship between Degree of Exposure to Hazards of Wood Work and Lung Function.

The American thoracic Association (ATS) defines obstructive disease as that in which maximal airflow (FEV) is disproportionately reduced with respect to the volumes of air a subject can exhale from full expiration (FVC).

In this study, the mean percentages of the predicted FVC for all age groups were within normal values. The percentage predicted means FEV_1 was however low for the subjects between 25 -34 years and those between 45 – 59 years old. The mean FEV_1/FVC for the wood workers across most of the age group were similarly normal except for the age groups 25 – 29years and 30 – 34years that had 74.16% and 73.82% of their predicted values respectively. The decrease could be as a result of those within that age involved more in jobs that have highest exposures to wood dust and chemicals (sanding and spraying). This pattern of lung function observed among the subjects in this study clearly depicts the obstructive pattern of lung function impairment. Lung function impairment was similarly reported by other researchers in Nigeria and elsewhere, amongst workers who were exposed to wood dust and other lung irritants in their work places (Bosan and Okpapi, 2004; Demissie, 2019). The reduction in FEV_1 in the older age groups may be explained by the fact that lung function reduces by age. The common respiratory symptoms associated with woodwork in this study include sneezing, regular blocked nose, runny nose, wheeze, breathlessness, tightness of chest and cough. Cough and sneezing were the two (2) most common occurring symptoms. However, wheeze occurred least among the wood workers. Similar finding was reported in past studies (Bosan and Okpapi, 2004; Chirdan and Akosu, 2004; Håkan *et al* , 2017). In addition, significant relationship between degree of exposure and lung function, and with prevalence of respiratory symptoms was also demonstrated in this study. Chirdan and Akosu, 2004; Bosan and Okpapi, 2004 also reported similar findings. This could be associated with similarities in the study group.

This study also observed that lung function impairment also increases with increase in degree of exposure. From the findings it has been shown that people who were exposed more have impairment of lung function more than those with lower exposure. Exposure to chemical used as coating and preservatives in the wood industry also cause lung function impairment. The wood dust and chemical formed produce damaging effect on the respiratory tract through allergic reaction as a direct irritant effect and or chronic inflammatory responses. As a result, respiratory symptoms such as chronic cough, corrhiza, breathlessness and wheezing manifest as found in the current study.

In conclusion, the study found obstructive lung function impairment among those who engaged more in sanding and spraying. The study also observed a negative correlation between degree of exposure to the hazards in the work place and lung function of the workers. As the exposure increases, lung function decreases. More attention should be paid to the provision of vents and use of mask in the wood work places.

Acknowledgement

The authors like to thank all the subjects that volunteered to participate in the study. We are also grateful to the technologists that assisted in the laboratory analysis.

REFERENCES

American Thoracic Society (1991). Lung function testing: Selection of referencevalues and interpretative strategies. Am Rev Respir Dis; 144; 1202-1218.

Anders B., Mikkelsen, Vivi S., Torben S. and Inger S. (2002). Determinants of wood dust exposure in the Danish furniture industry. Annals of Occupational Hygiene; 46:8; 673-685.

Bosan I.B. and Okpapi J.U (2004). Respiratory symptoms and ventilatory function impairment among workers in the savannah belt of northern Nigeria. Annals of African Medicine; 3(1):22-27.

Chirdan O.O. and Akosu J.T. (2004). Respiratory symtoms in workers at katako wood market, Jos, Plateau state, Nigeria. Journal of Community Medicine and Primary Health Care; 16:2; 30-33

Crompton G.K, Haslett C. and Chilvers E.R (1999). Diseases of the respiratory system. 19th ed. Davidson's Principles and Practice of Medicine. Churchill Livingstone England. Pp 242-259.

Hessel P.A., Herbert F.A., Melenka L.S., Yoshida K, Michaelchuk D, Nakaza M. (1995). Lung health in sawmill workers exposed to pine and spruce. Chest;108:642–6.

Kim, K. H., Jahan, S. A., & Lee, J. T. (2011). Exposure to formaldehyde and its potential human health hazards. Journal of Environmental Science and Health, Part C, 29(4), 277-299

Masoud N., Zeinab J., and Fatemeh K.S (2018). Functional disorders of the lung and symptoms of respiratory disease associated with occupational inhalation exposure to wood dust in Iran. Epidemiol Health; 2018: 40: e2018031.

Ministry of Manpower (2006). A semi-Quantitative Method to Assess Occupational Exposure to Harmful Chemicals. Occupational Safety and health Division, Singapore 059764

Montano D. (2014). Chemical and biological work-related risks across occupations in Europe: a review. Journal of occupational medicine and toxicology (London, England), 9, 28. doi:10.1186/1745-6673-9-28

Ratnasingam, J., Ioras, F., Tadin, I., Lim, T., & Ramasamy, G. (2014). Respiratory effects in woodworkers exposed to wood and wood coatings dust: A regional evaluation of South East Asian countries. J. Appl. Sci, 14, 1763-1768.

SAFE Work (2005). Health hazards of wood dust. Bulletin No. 238.

Shamssain M.H (1992). Pulmonary function and symptoms in workers exposed to wood dust. Thorax; 47:84–7.

Wood Dust study Group 1(1995). Summary of data reported and evaluation. Wood dust; 62: 35.

Wondu R.D., Dilnessa F., Tewodros G.M., and Elias M. (2019). Effect of Occupational Wood Dust on Pulmonary Function among Woodworkers in Jimma Town, Southwest Ethiopia, A Comparative Cross Sectional Study. Pulmonology and Respiratory Medicine; 8.8: 587-593

Workers Health Center Fact Sheet (2004). Wood dust. Occupational exposure standards for wood dust. National occupational health and safety commission..

Niger. J. Physiol. Sci. 35 (December 2020): 167 - 171
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Haematological Changes Associated with Newcastle Disease Vaccination in Chickens Using Gums from *Cedrela odorata* and *Khaya senegalensis* as Delivery Agents

Oyebanji V.O., Jarikre T.A., Jagun-Jubril A., Adeniran G.A. and Emikpe B.O.
Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Summary: Our previous ex-vivo and in vivo investigations have established immune-potentiating property of Khaya senegalensis and *Cedrela odorata* gums; however, the safety of the use of this gum combination in chicken has not been described. Hence this study evaluates the haematological profile of chickens vaccinated with Newcastle disease vaccine delivered through the oral and ocular routes using gums from *Cedrela odorata* and *Khaya senegalensis* as delivery agents. 252 one-day old chickens were grouped gum-vaccine oral (GVOR), vaccine oral (VOR), gum-vaccine ocular (GVOC), vaccine ocular (VOC), gum oral (GOR), gum ocular (GOC), no-gum-no-vaccine but challenged (NGNV/C), no-gum-no-vaccine unchallenged (NGNV/U). They were vaccinated on days 21 and 42 and challenged day 84. Blood samples were collected before first vaccination and at selected intervals afterwards. Analysis was done using one-way ANOVA with $P<0.05$ considered significant. Packed cell volume, total white cell count, heterocyte-lymphocyte ratios and platelet count varied insignificantly ($P>0.05$) throughout the period of observation across groups with no observable derangements. Hence, the absence of derangement in haematological indices from this study suggests that the dilution rate recommended from the ex-vivo study is safe for administration of Newcastle disease vaccine in chickens irrespective of the routes of delivery.

Keywords*: Mucilage, Adjuvants, Vaccine Delivery, Poultry, Newcastle Disease*

**Address for correspondence: get2theo@yahoo.com; Tel:+234-8062602408*

Received - July 2020; Accepted- October 2020

INTRODUCTION

Newcastle disease is an infectious disease of birds which depending on infecting strain could often result in high mortality. It is caused by a DNA virus belonging to the family Paramyxoviridae and genus-Avulavirus- the only member of that genus (Alexander 2000). The disease is endemic in most tropical regions of the world such as found in most West African countries where wild birds, free range chickens and backyard poultry maintains the re-circulation of the virus. The disease is also a threat to commercial poultry production as it often results in serious economic loss from mortalities and reduced egg production in laying flock. Although the disease is endemic in Nigeria, epidemic spikes have being reportedly observed from November to February and June-July each year (Okwor and Eze 2010).

Currently, there is no treatment for the disease but control measures had been by vaccination of flock. This is often done using lentogenic, avirulent, thermostable and Mesogenic strains. Most of these preparations come as either killed or live attenuated vaccines. However, live vaccine preparations mostly administered orally or aerosolized have been reported to elicit inconsistent humoral and cellular immune protective titers responses from flock to flock with reports of sub clinical infection commonly observed in laying flocks. The short duration of protection is one of the factors that often limit the use of this class of vaccines which in turn encouraged indiscriminate and self-designed vaccination schedules in farms with varying non-replicable results. Conversely, killed vaccines preparation often administered parenteral with oil adjuvants to improve immunogenicity and sustained response through a slow release mechanism have proven productive with prolonged duration of response. However, the limitation associated is the fact that it only elicits circulating antibody responses with a deficient cellular response (Okwor *et al* 2009, Okwor and Eze 2010).

Stemming from the above, our previous studies have focused on potentiating immune response to live attenuated vaccines along the mucosal route (oral or ocular) with more efficient delivery vehicle from natural bio-degradable sources such as plants. Hence, bioadhesives (gums) from Khaya senegalensis and Cedrela odorata were evaluated ex-vivo for possible mucoadhesive properties on trachea and intestine tissues of chicken, goats, sheep, cattle and pigs (Emikpe *et al* 2016). On subjection to haemagglutination (HA) test, these gums were found to possess strong haemagglutination property (log225) individually and in combined ratios (1:3- Khaya: Cedrela) which boosted the HA property of the gum-vaccine mixture when combined however with a suspected risk of haemagglutination under condition (Emikpe *et al* 2016).

This HA property from these gums has been suggested to stem from the presence of immunogenic large carbohydrates that make up the macromolecular structure of these gums such as Rhamnose, Lectins, Arabinose (Susuki *et al* 1994, Ingale and Hivrale 2013). Hence, checkerboard dilution was conducted to determine a concentration with minimal HA property while retaining the mucoadhesive and suspected potentiating property previous determined.

From the above procedure, a 1:8 dilution with HA property of Log22 was proposed safe in an in-vivo study. Therefore this study attempts to evaluate possible haematological derangement this dilution could cause over time when employed as a delivery agent for Newcastle disease under in-vivo conditions in chickens.

MATERIALS AND METHODS

Chickens: The study design had been earlier described (Oyebanji *et al* 2016). Briefly, two hundred and fifty-two (252) one-day old White Leghorn cockerels acquired from CHI® hatcheries, Ibadan, Nigeria were subdivided into 6 groups of 42 birds each. Namely: **A**: Gum vaccine oral (**GVOR**), **B**: Vaccine oral (**VOR**) **C**: Gum vaccine ocular (**GVOC**), **D**: Vaccine ocular (**VOC**), **E1**: Gum alone oral (**GOR**), **E2**: Gum alone ocular (**GOC**), **F1**: No Gum No Vaccine/Challenged (**NGNV/C**), **F2**: No Gum No Vaccine/Un-challenged (**NGNV/U**).

They were housed in a fumigated and well ventilated caged pen under standard brooding conditions provided at the experimental animal unit, University of Ibadan. Warmth, feed (Topfeeds®) and water were provided as required. All necessary vaccinations and treatments were given uniformly to the birds' aside the experimental vaccination which was done as subdivided into groups.

Haematology: For the haematological studies, 2ml of blood samples on each sampling day were collected into Ethylenediamine tetra acetate (EDTA) coated tubes (Seward ltd). Packed cell volume (PCV) was determined by Microhaematocrit method while the haemoglobin concentration was evaluated using the Sahli's (acid haematin) method (Benjamin 1978). The total erythrocyte counts and the total leucocyte counts were determined using the Neubar haemocytometer counting chamber while differential counts were determined from Geimsa stained blood smears. Mean corpuscular volume and mean corpuscular haemoglobin concentrations were calculated from PCV, HB and RBC values (Jain 1986).

Statistical analysis: Omnibus one-way ANOVA was used to analyze data value from the study. Any significant tests data value was subject to a post hoc test using Apriori Least Significant Difference Contrast (LSD). The latter was used because treatments groups were pre-grouped and compared as such. Samples were also pooled together and analyzed after each period i.e. post first vaccination, post second vaccination and post challenge because they are repeated sample of each measure.

Ethical Approval: All international protocols concerning animal studies were duly observed as well as institutional ethical guidelines for the in vivo study.

RESULTS

All Red cell parameters including the calculated values (MCV, MCH, and MCHC) during the period of observation showed insignificant ($P>0.05$) variation between each groups even during the post challenge period. Also white cells indices as well as differentials were insignificant ($P>0.05$) specifically, heterophils and lymphocyte counts as wells their ratios were insignificant. Heterocyte-lymphocyte ratio was insignificantly different ($P>0.05$) throughout the duration of the experiment. Results are presented in tables 1 & 2 below.

DISCUSSION

This study evaluates some selected haematological parameters of chicken vaccinated against Newcastle disease using gums from Cedrela odorata and Khaya senegalensis as delivery agent. This is following ex-vivo evaluations and suggestions of a suspected safe dilution dose (Emikpe *et al* 2016).

From this study, lack of significant derangement in haematological indices attests to the safety of the dilution rate used as vehicular delivery for Newcastle disease vaccines. Previous studies linking plants materials with haemagglutinating property have made exploration of natural products from plant less desirable due to extra efforts needed to purify such compounds. Such properties have been reported to be due to present of haemagglutinnin units or epitopes of the complex macromolecular structure of these plant materials (Kuku *et al* 2005, Torky 2016).

Therefore, it could be theoretically postulated that lower concentration i.e. dilutions, these compounds could be explored as seen with vaccine delivery without necessarily losing their efficacy as mucoadhesive or slow-release agent as posited in earlier ex-vivo studies (Emikpe *et al* 2016). Efficacy as mucoadhesive or slow-release agent as well as immunopotentiating agent in-vivo has been evaluated with evidence of the gum evoking an early and sustained response post-infection in groups where gums were used as delivery agent especially in the oral group (Oyebanji *et al* 2016). From this study with the haematological values within the safe range and comparable to the control group, it can be concluded that phytogenic mucoadhesives from Cedrela odorata and Khaya senegalensis used at 1:8 dilutions evokes no-haematological derangement in chickens in-vivo hence this recommended dilution rate should be used when this combination of this gum is used for vaccine delivery in chicken.

Funding: *This study was funded by the University of Ibadan McArthur Research Grant (Grant No. SRG/ FVM/2010/6A).*

REFERENCES

Alexander, D.J. (2000). Newcastle disease and other avian paramyxoviruses. Rev. Sci. tech. Off. Int. Epiz, 19(2), 443-462

Benjamin, M.M. (1978). Outline of Veterinary Clinical Pathology. Iowa State University Press, Ames, Iowa, USA. Pp 25-58, 103-104.

.

Table 1:
Packed Cell Volume of chicken in the different groups

Groups		Weeks post 1st vaccination				Weeks post 2nd vaccination.							Weeks post Challenge				
		1	2	3		1	2	3	4	5	6		1	2	3	4	5
A: GVOR		21.4 ±4.1	32.2 ±5.8	27.6 ±12.0		28.4 ±7.7	29 ±7.2	27.7 ±12.2	38 ±11.0	38.7 ±8.0	29.2 ±3.0		34.2 ±9.0	39.6 ±12.7	32.0 ±9.6	27.4 ±13.3	30.3 ±3.9
B: VOR		29.2 ±6.0	33 ±1.1	27.5 ±8.8		32.8 ±2.8	16 ±5.8	34 ±4.3	28 ±8.6	29.3 ±10.0	26.3 ±7.0		37.2 ±6.7	36.8 ±6.8	33.7 ±8.0	28.2 ±8.0	30.3 ±7.0
C: GVOC	1st Vaccination	29.3 ±7.0	32.6 ±7.1	30.8 ±7.9	2nd Vaccination	28.0 ±8.2	26.7 ±7.0	24.3 ±8.1	31.3 ±6.7	32.0 ±2.2	29.5 ±13.7	Challenge with live virus	38.4 ±6.9	37.0 ±8.0	31.7±8.3	28.4±11.0	31.3±5.8
D: VOC		28.8 ±2.5	33.6 ±10.1	30.3 ±9.7		26.5± 6.8	24.0± 13.0	22.5 ±9.0	31.3 ±9.0	32.3 ±6.2	32.0 ±12.8		30.6 ±12.0	36.0 ±7.0	30.7 ±9.0	29.8 ±14.1	29.7 ±2.0
E1:GOR		28.3 ±2.2	33.3 ±12.1	32.0 ±11.0		34.0± 6.5	25.3± 1.0	23.5 ±11.8	26.0 ±5.9	28.0 ±12.7	25.3 ±9.1		35.2 ±3.0	32.3 ±7.9	24.8 ±6.7	27.3 ±5.9	27.8 ±6.8
E2:GOV		28.0 ±2.9	33.3 ±9.6	32.0 ±4.5		30.0± 8.1	29.0± 5.4	24.5 ±2.9	19.0 ±6.8	29.0 ±4.8	24.5 ±2.0		34.2 ±11.1	33.2 ±11.4	23.4 ±10.2	26.7 ±7.8	26.3 ±6.0
F1: NGNV/C	--	31.0 ±4.2	32.0 ±11.1	28.0 ±6.8		26.7± 10.1	15.0± 1.0	24.3 ±10.0	26.0 ±9.4	32.6 ±10.7	28.0 ±11.7		30.5 ±8.1	29.5 ±6.8	28.8 ±6.0	24.5 ±1.9	28.3 ±3.9
F2: NGNV/U	--	31.0 ±3.0	32.0 ±4.3	28.0 ±6.0	--	26.1± 12.0	18.0± 3.2	23.4 ±9.8	26.7 ±8.5	31.0 ±5.0	27.8 ±6.8	--	33.4 ±12.9	36.9 ±8.5	33.0 ±7.9	28.4 ±1.8	29.7 ±2.2

Legend: GVOR: Gum-Vaccine Oral Group, VOR-Vaccine Oral Group, GVOC: Gum-Vaccine Ocular Group, VOC: Vaccine ocular group, GOR: Gum Oral alone Group, GOC: Gum Ocular alone Group, NGNV/C: No-Gum-No-Vaccine/Challenged Group, NGNV/U: No-Gum-No-Vaccine/Unchallenged Group

Table 2:
Total white cells count, selected differentials and heterocyte-lymphocyte ratio.

		A: GVOR	B: VOR	C: GVOR	D: VOR	E1: GOR	E2: GOC	F1: NGNV/C	F2: NGNV/U
Wk 1 Po 1st Vacc.	TWBC	15.1±12.0	15.2±22.0	16.9±16.1	17.1±10.9	16.6±5.6	16.1±2.9	14.4±24.4	14.4±13.1
	Heterophil	33.4±1.4	31.6±2.8	28.5±2.9	33.8±2.7	25.7±8.3	37.0±6.2	30.8±7.1	30.7±7.4
	Lymphocyte	59.2±11.0	61.8±13.0	75.3±12.7	59.2±11.7	68.3±10.4	56.3±15.1	63.0±9.1	60.0±9.7
	Het/Lym	0.56±0.31	0.51±1.16	0.38±0.21	0.57±1.0	0.38±0.30	0.68±0.33	0.49±0.42	0.51±0.23
	Platelets x10^3	205.0±20.0	188.0±23.0	145.8±12.7	214.8±21.0	209.7±34.1	155.0±24.7	168.5±28.1	166.0±31.0
Wk 2 Po 1st Vacc.	TWBC x10^3	15.6±6.9	16.7±10.0	16.9±2.2	14.8±11.3	16.9±8.1	14.812.1	16.7±11.6	15.810.0
	Heterophil	30.4±16.2	32.812.8	35.4±6.8	31.6±10.8	33.7±14.0	25.0±18.9	36.5±17.6	35.6±16.3
	Lymphocyte	63.6±11.3	61.8±14.3	59.8±17.2	61.6±8.7	59.3±26.7	68.3±6.8	67.8±4.3	66.5±4.7
	Het/Lym	0.48±0.12	0.53±0.61	0.59±0.81	0.51±1.02	0.57±0.91	0.37±0.23	0.54±1.0	0.54±1.0
	Platelets x10^3	169.8±30.8	182.8±24.2	181.6±18.9	145.6±28.4	206.0±23.7	262.7±34.3	155.0±16.8	156.3±19.0
Wk 2 Po 2nd Vacc.	TWBC x10^3	20.4±14.2	16.6±8.12	22.0±14.4	16.6±11.9	19.3±2.01	19.9±10.2	15.8±11.9	15.4±10.9
	Heterophil	43.7±22.1	49.5±11.9	35.7±18.8	38.5v12.7	30.0±10.1	30.1±13.6	43.5±21.3	40.4±18.2
	Lymphocyte	59.3±26.5	44.5±20.0	57.7±23.6	55.0±25.4	63.3±10.4	64.5±23.1	48.2±16.8	48.8±19.1
	Het/Lym	0.74±0.22	1.11±0.78	0.62±0.11	0.7±0.22	0.47±0.12	0.47±0.11	0.9±0.12	0.83±0.9
	Platelets x10^3	176.3±40.1	131.0±38.8	192.7±26.9	206.0±32.9	213.7±22.0	188.5±19.0	188.0±34.8	185.0±12.9
Wk 6 Po 2nd Vacc.	TWBC x10^3	20.4±10.1	23.6±9.6	20.5±11.6	18.9±8.9	20.5±8.6	20.4±10.6	20.9±12.1	19.4±3.4
	Heterophil	40.4±12.4	36.3±22.1	28.5±23.1	34.6±18.5	37.5±12.2	38±17.6	36.8±17.0	35.3±11.0
	Lymphocyte	51.8±22.1	55.3±17.9	55.8±24.2	57.2±32.8	54.8±12.8	54.0±23.8	55.0±31.0	54.9±12.0
	Het/Lym	0.78±0.53	0.66±0.31	0.51±0.40	0.61±0.99	0.68±0.12	0.7±0.31	0.67±0.23	0.64±0.11
	Platelets x10^3	279.6±53.1	297.0±23.9	230.3±49.2	272.8±40.0	251.3±37.6	273.8±36.1	271.9±18.8	267.9±26.5
WK 2 PoC	TWBC x10^3	16.3±4.5	16.9±6.9	16.3±8.1	17.4±2.3	16.5±11.0	16.2±6.9	15.0±6.8	14.9±7.0
	Heterophil	34.2±21.0	27±13.3	29.3±12.0	37.6±26.3	31.3±21.0	33.3±19.6	36.5±16.8	36.2±22.5
	Lymphocyte	58.8±26.8	66.3±33.4	64.0±27.3	56.2±21.0	66.3±28.1	62.1±24.9	56.0±11.0	55.4±24.1
	Het/Lym	0.58±0.21	0.41±0.30	0.46±0.21	0.67±0.41	0.47±0.21	0.54±0.21	0.66±0.21	0.65±0.33
	Platelets x10^3	257.0±66.0	214.0±49.2	165.2±24.4	164.4±51.0	176.3±28.9	180.1±67.2	252±23.0	236±21.2
Wk 5 PoC	TWBC x10^3	16.3±1.9	16.4±3.4	13.2±1.3	14.1±2.7	12.6±8.2	13.7±2.1	15.1±2.3	14.5±6.7
	Heterophil	27.7±11.9	22.3±16.2	21.7±9.7	26.3±12.3	27.5±13.5	25.9±12.5	27.8±11.7	27.1±16.8
	Lymphocyte	66.3±22.0	71.3±23.4	72.3±23.0	66.7±21.0	65.0±23.7	64.0±19.3	65.8±14.4	63.5±11.1
	Het/Lym	0.42±0.11	0.31±0.12	0.3±0.15	0.39±0.20	0.42±0.23	0.4±0.12	0.42±0.12	0.43±0.16
	Platelets x10^3	230.0±29.5	164.0±34.7	178.3±23.7	199.0±34.9	154.2±39.1	150.3±23.7	162.5±45.3	166.8±24.6

Legend: GVOR: Gum-Vaccine Oral Group, VOR-Vaccine Oral Group, GVOC: Gum-Vaccine Ocular Group, VOC: Vaccine ocular group, GOR: Gum Oral alone Group, GOC: Gum Ocular alone Group, NGNV/C: No-Gum-No-Vaccine/Challenged Group, NGNV/U: No-Gum-No-Vaccine/Unchallenged Group.

Emikpe, B.O., Oyebanji, V.O., Odeniyi, M.A., Salaam, A.M., Oladele, O.A., Jarikre, T.A., and Akinboade, OA. (2016). Ex-vivo evaluation of the mucoadhesive properties of Cedrela odorata and Khaya senegalensis gums with possible applications for veterinary vaccine delivery. SpringerPlus (5): 1289. doi:10.1186/s40064-016-2948-0.

Ingale, A.G, and Hivrale, AU. (2013). Plant as a plenteous reserve of lectin-a review. Plant Signal Behav. Dec; 8(12): e26595.

Jain, N.C. (1986). Schalm's Veterinary Haematology, 4th ed. Lea and Febriger Philadelphia. Pp.32-35.

Kuku, A., Agboola, F., and Aboderin, A. (2005). Purification and Characterisation of a Lectin from the seeds of Psophocarpus palustris. Pak. J. Biol. Sci., 8 (12): 1667-1671

Okwor, E.C, Eze, D.C. (2010). The annual prevalence of Newcastle disease in commercial chickens reared in South Eastern Savannah zone of Nigeria. Res. J.Poult. Sci. 3: 23-26.

Okwor, E.C., Eze, D.C. and Uzuegbu, M.O. (2009). Effect of storage condition on the potency of Newcastle disease vaccine la sota. Int. J. Poult. Sci., (8); 999-1002.

Oyebanji, V.O., Emikpe, B.O., Oladele, O, Odeniyi, MO, Salami, A, Osowole, O.I., Kasali, O.B., and Akinboade, O.A. (2016) Evaluation of immune response in challenged chickens administered with Newcastle disease vaccine using gums from *Cedrela odorata* and *Khaya senegalensis* as delivery agents. J. of immunoas. and Immunochem. http://dx. doi.org/10.1080/ 153 21819 .2016 .1273237

Susuki, M, Takatsuki, F., Maeda, Y.Y., Hamuro, J, *et al*. (1994). Lentinan-rationale for development and therapeutic potential. Clin. Immunother.; 2: 121–5.

Torky, Z.A. (2016). Antiviral Activity of Euphorbia Lectin Against Herpes Simplex Virus 1 and its Antiproliferative Activity Against Human Cancer Cell-Line. J Antivir Antiretrovir 8:107-116. doi: 10.4172/1948-5964.1000142.

Niger. J. Physiol. Sci. 35 (December 2020): 173 - 179
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Hepato-Genoprotective Activities of Methanol Extract of The Stem Bark of *Adansonia Digitata* LINN. In Wistar Rats Challenged with Sodium Arsenite

Adegoke A.M., Gbadegesin M.A., *Odunola O.A

Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Summary: Arsenic exposure is an issue of concern in developing countries, consequently leading to arsenicosis which has been implicated in the development of cancers. There are various traditional medicinal applications of the stem bark of Adansonia digitata (SBAD). The focus of this study was to explore the hepatoprotective and antigenotoxic properties of methanol extract of SBAD (MESBAD) against sodium arsenite - induced toxicities in Wistar rats. Phytochemical investigation of the extract was done according to established procedures. Hepato-genoprotective properties were assessed using the liver function tests with histology and micronucleus induction assay respectively. Thirty (30) rats distributed into six groups (five rats each) were used for the experiment. Negative control (distilled water and rat pellets only), positive control [2.5 mg/kg body weight of sodium arsenite (SA)]. Test animals were challenged with SA and treated with 300 or 400 mg/kg body weight of MESBAD. Phytochemical analysis showed that MESBAD possess high concentration of alkaloids, saponins, flavonoids and total polyphenols. The SA increased the activities of ALP, GGT and the frequency of micronucleated polychromatic erythrocytes (nMPCEs) induced in rat bone marrow when juxtaposed with the negative control. Treatment with MESBAD significantly ($p<0.05$) reduced these parameters, histological examination of the liver showed that MESBAD reduced the severe portal and central venous congestion induced by SA, methanol extract of the stem bark of Adansonia digitata mitigates SA-induced toxicities probably through radical scavenging activities.

Keywords*: Adansonia digitata, hepatotoxicity, genotoxicity, methanol extract, phytochemical analysis, sodium arsenite*

**Address for correspondence: ronodunola@yahoo.com; Tel:* +234 8023387152

Manuscript Received – October, 2019; Accepted - July, 2020

INTRODUCTION

Arsenic is abundant in the earth's crust but occur in small quantities in rock, soil, water and air. It is a common environmental pollutant, as it is the case in Bangladesh with arsenic contaminated groundwater. Exposure to arsenic occur via inhalation, absorption through the skin and majorly by ingestion of arsenic contaminated food and water (Fulladosa *et al* ., 2007). Long-term arsenic exposure can lead to several sicknesses, including, arterial hypertension and cardiovascular disease, skin lesions, pulmonary disease etc (Smith *et al* ., 2000; Flanagan *et al* ., 2012), and different cancers, such as cancer of the lung, bladder, kidney and skin (Chen *et al* ., 1992; Smith *et al* ., 1998).

Natural products, on the other hand, play crucial roles in the therapy and care of various ailments. The high cost and toxicities associated with synthetic drugs make the search for potent antitoxic agents from plant origin a necessity. *Adansonia digitata* (Linn) is a member of the *Bombacaceae* family, commonly known as Baobab, Monkey Bread Tree, Cream of Tartar Tree, etc. The tree is mostly found in the savanna regions of the world (Keay, 1989). There are reports about the folkloric uses of *Adansonia digitata* both for dietary and several medicinal purposes (Chadare *et al* ., 2009). Some of the reported medicinal uses are as antimalaria, treatment of intestinal problems and skin disorders, anti- inflammatory, antipyretic and analgesic (Sidibe and Williams, 2002; De Caluwe *et al* ., 2010; Ramadan *et al* ., 1994; Ajose, 2007; Karumi *et al* ., 2008). The stem bark has been reported to possess antibacterial properties (Yushua'u *et al* ., 2010; Atawodi *et al* ., 2003), and hypoglycemic activity (Tanko *et al* ., 2008). The stem bark posses the alkaloid "Adansonin" which is suggested to be the potent active component for the treatment of malaria and other pyrexia (Sidibe and Williams, 2002). The antiviral and anti-trypanosomal activities of baobab extracts have also been reported (Vimalanathan and Hudson, 2009; Sulaiman *et al* ., 2011).

There is inadequate information in literature on the hepatoprotective and antigenotoxic activities associated with the methanol extract of the stem bark of *Adansonia digitata* (MESBAD). This study was designed to explore the hepato-genoprotective potentials of MESBAD against sodium arsenite (SA) - induced toxicities in Wistar rats.

MATERIALS AND METHODS

Plant material and preparation of extract: Leaves and fruits of baobab were used to identify and authenticate (FHI NO.109859) the forest tree and the voucher number stored at Forestry Research Institute herbarium, Jericho, Ibadan Nigeria. Fresh stem bark were harvested and dried at room temperature, it was thereafter milled and extracted

with 70% methanol for 72 hrs. The extract obtained was subjected to concentration in a rotary evaporator at 30 – 40 ^{o}C, lyophilized and kept for use (Adegoke *et al* ., 2017).

Experimental animals and treatments: Thirty (30) male Wistar rats (100-150g) were procured and kept in the animal house, Biochemistry Department, University of Ibadan, Nigeria at 29 ± 2 ^{o}C, 12 hours light/dark cycle, maintained on water and rat feeds *ad libitum* (Ladokun Livestock Feeds Limited, Ibadan, Nigeria). All the animals used for this study were handled in conformity to the guide for the care and use of experimental animals, as stipulated by the National Institute of Health (NIH publications number 85–93 revised in 1985).

The rats acclimatized for seven (7) days prior to the commencement of the study. Rats were distributed into six groups (five animals each). **Group 1** Negative control, received water and standard pelleted diet only. **Group 2** was given 400 mg/kg body weight MESBAD, **Group 3** was administered 400 mg/kg MESBAD + SA. **Group 4** received 300 mg/kg MESBAD + SA, **Group 5** was given 300 mg/kg MESBAD alone, **Group 6** was administered SA only. Sodium arsenite was administered at 2.5 mg/kg body weight (10 % oral LD_{50}) (Preston *et al* ., 1987). All treatments were done by gavage for 14 days.

Reagents and kits: Kits for alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) were procured from Randox Laboratories, UK. Sodium arsenite ($NaAsO_2$) was product of BDH chemicals Ltd poole England, other chemicals and reagents used for this study were of analytical grade, and were products of Sigma Chemical Co. St. Louis, MO., USA.

Phytochemical investigations: The MESBAD was subjected to the phytochemical screening carried out according to standard procedures, to test for polyphenols, flavonoids, alkaloids, tannins, saponins, carotenoids, Terpenoids, Oxalate, Anthocyanins, steroids, protease inhibitors and cyanogenic glycosides.

Determination of Total Phenol: The method of Singleton *et al* (1999), was employed in the determination of total phenol content.

Determination of Total Flavonoid: Total flavonoid content was evaluated using a colorimeter assay described by Bao (2005).

Determination of Alkaloid: Alkaloid content was determined by the method described by Harborne (1973).

Determination of Tanins: Tanins content was determined by employing the method described by Van-Burden and Robinson (1981).

Determination of Saponins: Saponins content was determined according to the method described by Obadoni and Ochuko (2001).

Determination of Carotenoids: Carotenoids content was evaluated according to the method described by Harbone (1998).

Determination of Terpenoids: Terpenoids content was evaluated according to the method earlier described by Ejikeme *et al* (2014).

Determination of Oxalate: Oxalate was evaluated by using the method previously reported by Ejikeme *et al* (2014); Munro and Bassir (1969).

Determination of Anthocyanin content: Anthocyanin content was determined using a method earlier described by Connor *et al* (2002).

Determination of Steroid: Analytical method used is according to the method of Ejikeme *et al* (2014).

Determination of Protease inhibitor: Activity of protease inhibitor against protease was assayed according to the procedure described by Kunitz (1947).

Determination of Cyanogenic Glycoside: Cyanogenic glycoside was determined according to the method earlier described by Amadi *et al* (2004).

Liver function enzymes assays

γ-glutamyl transferase (γ-GT) activity. The γ-GT was evaluated in the serum using the reconstituted γGT diagnostic reagent following the previously described method of Szasz (1974).

Alkaline phosphatase (ALP) activity: The ALP was determined according to the optimized recommended method of the Deutsche Gesellschaft fur Klinische Chemie (DGKC, 1972).

p-nitrophenylphosphate + H_2O ⟶ ALP phosphate + p-nitrophenol (Tietz *et al* ., 1983).

Liver histological examination: The liver sections were fixed in 4 % p-formaldehyde and cleansed in phosphate buffer for 12 hours pH 7.4 at 4 ^{o}C. After drying out, the tissue was immersed in paraffin, and then cut into segments; the sections were stained with haematoxylin–eosin dye and viewed under a microscope.

Micronucleus (MN) assay : Rat femurs were excised and each bone marrow aspirated with a needle and syringe. Microscopic slides were prepared from the bone marrows according to the procedure previously described by Matter and Schmid, 1971. Slides prepared were fixed in methanol, air-dried and then pre-treated with May-Grunwald solution, thereafter air-dried again. These slides were further stained in 5% Giemsa solution and then induced in phosphate buffer for about 30 seconds. It was thereafter rinsed in distilled water and air-dried again. The slides were mounted and scored for micronucleated polychromatic erythrocytes (MPCEs) under a microscope, according to the standard procedure at a specified X40 magnification.

Data analyses: Results are expressed as mean ± Standard deviation. The differences between the groups were analyzed using one-way analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 10.0.1. $p < 0.05$ was considered statistically significant for differences in means.

RESULTS

Phytochemical analysis of methanol extract of the stem bark of *Adansonia digitata* (MESBAD): There is abundant of alkaloids present in MESBAD, while saponins, flavonoids and total polyphenols are present in significant amounts (Figure 1). Alkaloids are abundant with the highest value of 330.00±0.00 mg/100g followed by saponins (153.33±2.89 mg/100g). Flavonoids 121.67±2.89 mg/100g and total polyphenols 121.67±2.89 mg/100g.

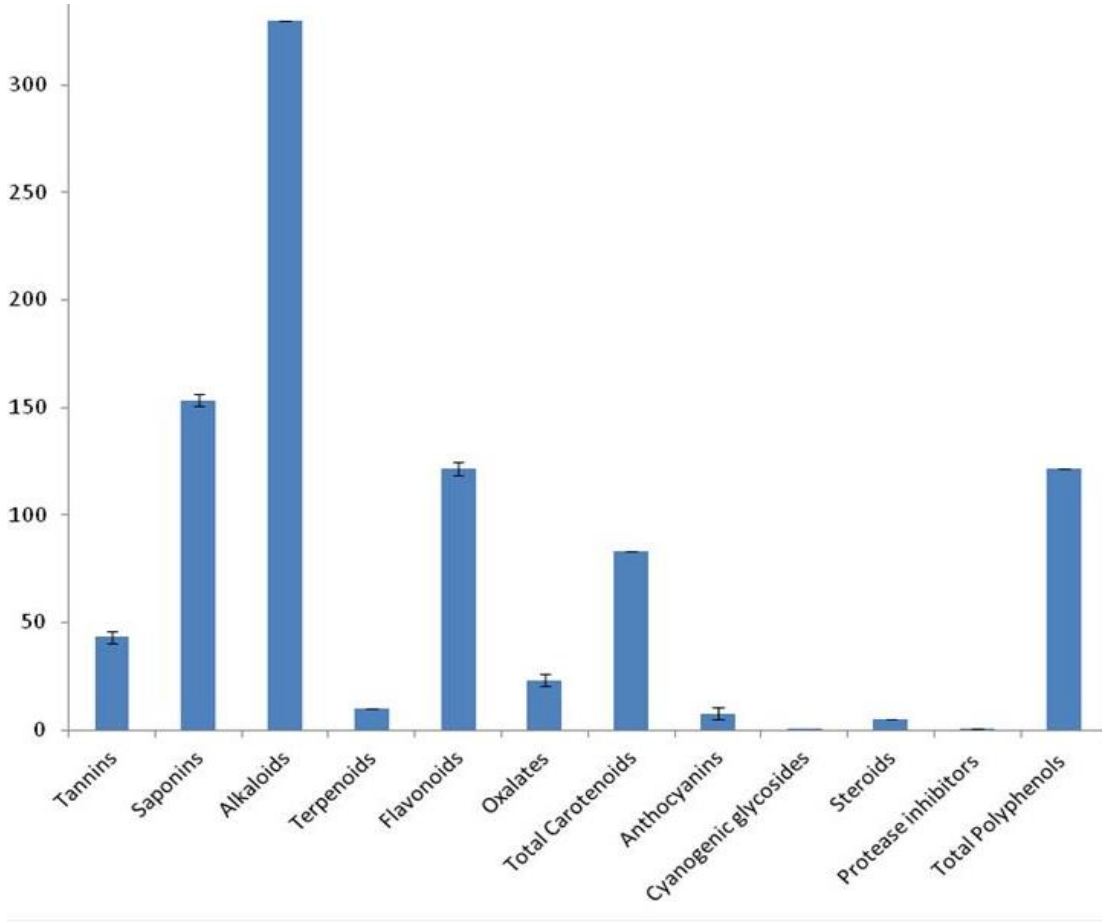


Figure 1:
The concentration of some phytochemicals in methanol extract of the stem bark of *Adansonia digitata.* Each bar represents mean ± S.D of 3 determinations * indicate a significantly high amount ** indicate abundance

Table 1:
The effect of MESBAD *and/or* SA on weight of liver and relative weights of liver of the treated rats. (values are mean ±SD)

Group	WoL (gs)	RWoL
Distilled water only	6.28±0.85	4.64±0.54
400mg/kg extract	7.00±1.60	4.26±0.78
400mg/kg extract+SA	6.38±1.13	4.06±0.48
300mg/kg extract+SA	6.00±0.21	4.48±0.79
300mg/kg extract	5.06±0.61	4.09±0.20
Sodium arsenite alone	5.54±0.33	3.03±0.11

Values are expressed as mean +or – stdev. a = the mean difference is significant (p<0.5) when compared with group a. gs = grammes

Hepatoprotective activities of MESBAD in Wistar rats challenged with SA. : Hepatoprotective effect of the stem bark of *Adansonia digitata* was investigated by assessing the activities of serum enzymes; γ-glutamyltransferase (γGT) and alkaline phosphatase. Administration of SA resulted in an increase in the mean serum ALP and γGT activities when compared with negative control, which received distilled water only (Table 2). The MESBAD alone at the doses of 300 and 400 mg/kg body weight did not induce significant ($p<0.05$) higher level of serum ALP and γGT activities when compared with the negative control.

Table 2: The effect of MESBAD *and/or* SA on liver function enzymes [Alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT)] of treated rats.

Group	ALP	γGT
Distilled water only	22.52±1.59	28.65±16.24
400 mg/kg extract	20.68±15.45	31.51±9.71
400 mg/kg extract + SA	31.72±0.00	30.16±10.64
300 mg/kg extract + SA	35.86±1.95	38.26±0.82
300 mg/kg extract alone	34.48±0.00	28.42±21.29
Sodium arsenite alone	42.07±39.32	40.87±3.19

Data are expressed as mean ± sd. (n=5), DW=Distilled water, SA=Sodium arsenite.

Mean serum activities of ALP and GGT of rats after treatment with MESBAD or SA

The histological assessment of the liver cells gave robust information that buttressed the serum enzyme activities (Figure 2). There was severe portal and central venous congestion in the liver of rats given SA only (positive control). No visible lesion in the negative control, the groups that received 400 mg/kg extract+SA and the group administered 300 mg/kg extract alone (groups 1, 3 and 5 respectively).

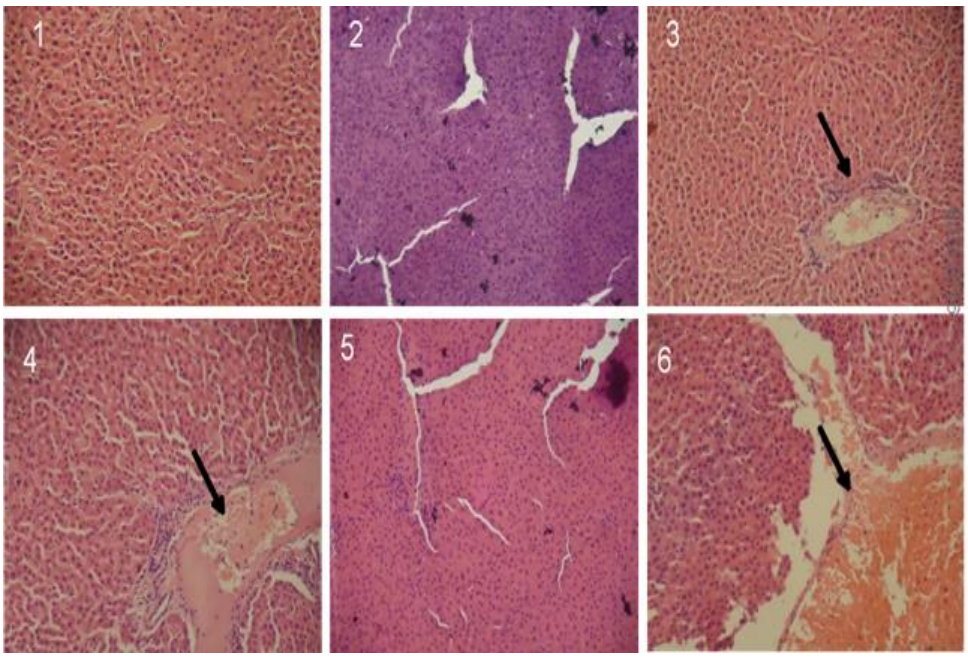


Plate 1:
Representative photomicrograph of the of the liver of rats treated with sodium arsenite and/methanol extract of the stem bark of *Adansonia digitata.* (Magnification= x400). 1. Distilled water only, normal hepatocytes, no visible lesion. 2. 400mg/kg MESBAD, mild portal congestion 3. 400mg/kg MESBAD+SA, normal hepatocytes. 4. 300mg/kg MESBAD+SA, very mild portal congestion, diffuse periportal cellular infiltration by mononuclear cells. 5. 300mg/kg MESBAD alone, no visible lesion 6. SA alone, severe portal and central venous congestion.

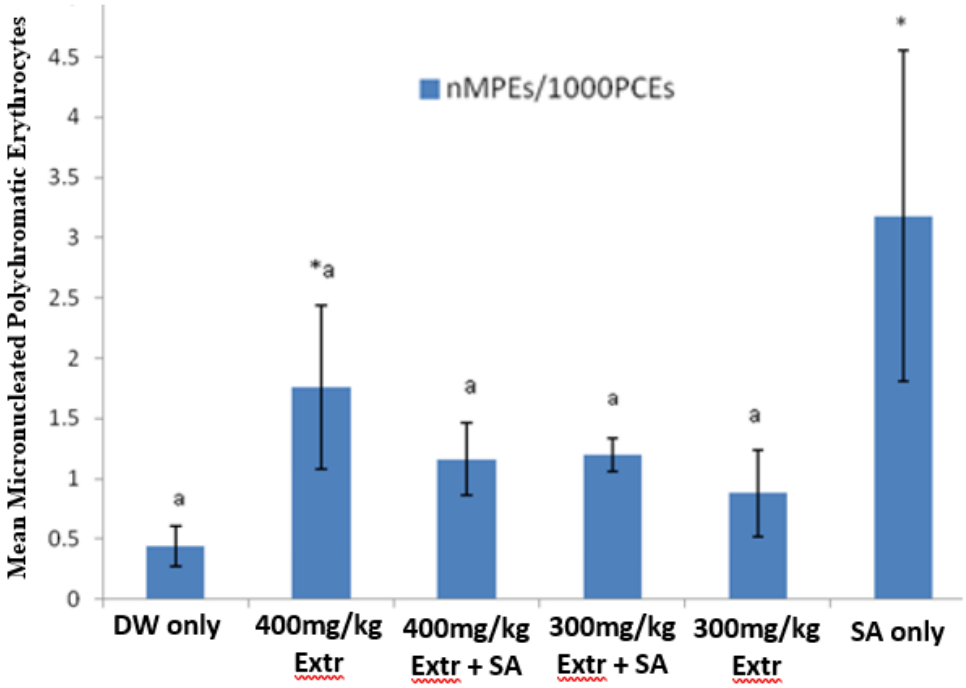


Figure 2:
Mean number of micronucleated polychromatic erythrocytes (nMPCEs) of the experimental animals after treatment with *Adansonia digitata* stem bark extract or sodium arsenite (SA). Data are expressed as mean ± sd. (n=5). DW=Distilled water, SA=Sodium arsenite. * mean difference is significant ($p< 0.05$) when compared with DW only group, [a] mean difference is significant ($p< 0.05$) when compared with SA only groups

There was mild portal congestion in group 2 administered 400 mg/kg of extracts only. However, there is very mild portal congestion and diffuse periportal cellular infiltration by mononuclear cells in group 4 administered 300mg/kg MESBAD+SA, and severe portal and central venous congestion in the group administered SA alone.

Antigenotoxic activity of MESBAD in rats challenged with SA.

The frequency of mPCEs per 1000 PCEs recorded in the bone marrow cells of rats (Figure 3) is significantly higher ($p < 0.05$) in the group that received SA alone (groups 6) when juxtaposed with the negative control (group 1). Treatments with the MESBAD led to a significant ($p<0.05$) reduction in the frequency of mPCEs scored in the groups that received both MESBAD and SA when juxtaposed with the group given SA alone. In other word, when juxtaposed with the positive control, given SA only, MESBAD significantly ($p<0.05$) reduced the number of mPCEs when co-administered with SA (groups 3 and 4).

DISCUSSION

Sodium arsenite (NaAsO2) is a known human carcinogen, its cancer-causing potential has been reported by the International Agency for Research on Cancer (IARC) (Hughes *et al* ., 2011; IARC, 1973). Some reports have also shown that sodium arsenite is genotoxic and hepatotoxic

(Mallick *et al* ., 2003; Odunola *et al* ., 2007, Gbadegesin *et al* ., 2014; Adegoke *et al* ., 2017). Adansonia digitata is a well-known forest tree with many uses in folk medicine; it is employed in the management of various diseases in certain parts of Africa. The current study investigates the hepato-genoprotective activities of MESBAD in rats challenged with sodium arsenite.

The health benefits derived from plants are numerous and they have severally been associated with many phytochemicals found in plants, phytochemicals in plants have been reported to be responsible for their medicinal properties (Hill, 1952). The MESBAD was therefore subjected to the phytochemical screening. In this study, various phytochemicals and bioactive compounds present in MESBAD were analyzed. The analysis showed that alkaloids are present in abundance, also saponins, flavonoids and polyphenols in an appreciable amount. Some of these phytochemicals have been found in many plants and had been reportedly used for medicinal purposes. The concentration of some phytochemicals in MESBAD is in this order:- Alkaloids > Saponins > Flavonoids > Total polyphenol > Tanins. Most phytoconstituents scavenge free radicals and therefore reduce oxidative stress. Examples of phenolic compounds are flavonoids and tannins, these compounds act as antioxidants or free radical scavengers. Also, terpenoids, as vitamins, play vital roles as regulators of metabolism and as antioxidants (Nair *et al* ., 1998; Agbafor *et al* ., 2014). Alkaloids possess several therapeutic activities such as antimalarial, antiasthmatic and anticancer properties as reported by Kittakoop *et al* . 2014. Flavonoids possess antioxidant, anti-inflammatory, anti allergic, anti carcinogenic, anti microbial, hepatoprotective and anti viral properties (O'Neil *et al* ., 2000; Ajuru *et al* ., 2017). Phenolic compounds prevent chronic diseases such as cardiovascular disease, some cancers, neurodegenerative disease, and also diabetes (Scalbert *et al* ., 2005). Saponins also possess anticancer properties, immunomodulatory properties, cell cycle regulation and cholesterol regulatory property (Jimoh and Oladiji, 2005). Alkaloids, flavonoids and saponins are known to possess hepatoprotective activities (Reddy *et al* ., 2015).This indicates that the extract is a good drug candidate against oxidative stress and various diseases associated with it.

The liver and relative liver weights of the treated rats were taken and compared, to establish any direct effect of the treatments on the liver and body weights, we noticed a decrease in WoL of rats treated with SA and also that of 300 mg/kg extract only, when colligated with the negative control administered distilled water only. However, the group administered sodium arsenite and the other test groups did not show significant changes on the relative weight of liver (RWoL) to body weight across board, when juxtaposed with the negative control, which was administered distilled water only.

Administration of SA led to an increase in the mean serum ALP and γGT activities when juxtaposed with negative control, which received distilled water only. The extract reduced the elevated ALP and γGT close to that of the negative control. This indicates a mild induction of hepatotoxicity in the liver cells, but the simultaneous administration of the extract reduced the values close to that of the negative control. The extract at the doses of 300 and 400 mg/kg body weight did not produce any significant higher levels of serum ALP and γGT activities. The above results are corroborated and better portrayed by histological analysis of the liver obtained from animals in each group. Histological analysis revealed that, there was severe portal and central venous congestion in the rats given SA only. No visible lesion in the negative control and the groups that received 400mg/kg extract+SA and 300 mg/kg extract alone. There was mild portal congestion in the group given 400 mg/kg of extracts only. However, there is very mild portal congestion and diffuse periportal cellular infiltration by mononuclear cells in group 4 administered 300mg/kg MESBAD+SA, and severe portal and central venous congestion in the group administered SA alone. The results indicate that SA caused hepatotoxicity and degeneration of the hepatocytes. Also, MESBAD possess hepatoprotective activities against sodium arsenite-induced hepatotoxicity.

The frequency of mPCEs per 1000 PCEs scored in rat bone marrow is an index for the measurement of genotoxicity and antigenotoxic potential of substances and compounds. Our study revealed that the frequency of mPCEs was higher significantly in the rats that received sodium arsenite alone, when colligated with the negative control. Administration of the extract led to a significant reduction in the frequency of mPCEs scored in the groups that received the extract and sodium arsenite when juxtaposed with the group that received sodium arsenite only, the extract significantly reduced the frequency of mPCEs when it was given along with sodium arsenite. This study therefore, revalidates the genotoxic properties of sodium arsenite and also provided proofs about the antigenotoxic properties of the methanol extract of the stem bark of Adansonia digitata against sodium arsenite-induces genotoxicity in rats.

From the results, it could be inferred that SA possess hepatotoxic and genotoxic effect. The result also show the protective role of MESBAD on SA-induced toxicity. Methanol extract of the stem bark of Adansonia digitata showed potent hepatoprotective and angenotoxic activities in rats challenged with sodium arsenite. These observed activities may be attributed to the phytochemicals present in the extract. Future research is required to be targeted at isolation, purification and characterization of the active principle in the extract.

REFERENCES

Adegoke, A. M., Gbadegesin, M. A., Otitoju, A. P. and Odunola, O. A. (2017). Hepatotoxicity and Genotoxicity of Sodium Arsenite and Cyclophosphamide in Rats: Protective Effects of Aqueous Extract of Adansonia digitata L. Fruit Pulp. British Journal of Medicine and Medical Research Vol.8. No.11: 963 – 974.

Agbafor, K. N., Ogbanshi, M. E. and Akubugwo, E. I. (2014). Phytochemical screening, hepatoprotective and antioxidant effects of leaf extracts of Zapoteca portoricensis. Advances in Biological Chemistry 4: 35-39.

Ajose, F. O. A. (2007). Some Nigerian plants of dermatologic importance. Int. J. Derm. 46 (suppl 1): 48-55.

Ajuru, M. G., Williams, L. F. and Ajuru G. (2017). Qualitative and Quantitative Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger

Delta Region of Nigeria. Journal of Food and Nutrition Sciences. Vol. 5, No. 5. pp. 198-205.

Amadi, B. A., Agomuo, E. N. and Ibegbulem, C. O. (2004). Research Methods in Biochemistry. Supreme Publishers, Owerri. 90-115.

Atawodi, S. E., Bulus, T., Ibrahim, S., Ameh, D. A., Nok, A. J., Mamman, N., Galadima, M. (2003). In Vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. Afr. J. Biotechnol. 2: 317-321.

Bao, J. S., Cai, Y., Sun, M., Wang, G. and Corke, H. (2005). Anthocyanins, flavonols, and free radi-cal scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability. Journal of Agricultural and Food Chemistry 53: 2327-2332.

Chadare, F. J., Linnemann, A. R., Hounhouigan, J. D., Nout, M. J. R., Van Boekel, M. A. J. (2009). Baobab Food Products: A Review on their Composition and Nutritional Value. Crit. Rev. Food Sci. Nutr. 49: 254-274.

Chen, C. J., Chen, C., Wu, M. and Kuo, T. (1992). Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer: 66:888-92.

Connor, A. M., Luby, J. J. and Tong, C. B. S. (2002). Variability in antioxidant activity in blueberry and correlations among different antioxidant activity assays. Journal of American Society for Horticultural Science 127: 238-244.

De Caluwé, E., Halamová, K. and Van Damme, P. (2010). Adansonia digitata L. – A review of traditional uses, phytochemistry and pharmacology. Afrika focus — Volume 23, Nr. 1. pp. 11-51

Deutsche Gesellschaft für klinische Chemie (DGKC) (1972). Empfehlungen der deutschen Gesellschaft für Klinische Chemie Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.) Z Klin Chem Klin Biochem: 10:182-92.

Ejikeme, C. M., Ezeonu, C. S. and Eboatu, A. N. (2014). Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. European Scientific Journal 10(18): 247–270.

Flanagan, S. V., Johnston, R. B. and Zheng, Y. (2012). Arsenic in tube well water in Bangladesh: health and economic impacts and implications for arsenic mitigation. Bulletin of the World Health Organization; Type: Policy & practice Article ID: BLT.11.101253

Fulladosa, E., Murat, J. C., Bollinger, J. C. and Villaescusa, I. (2007) Adverse effects of organic arsenical compounds towards Vibrio fischeri bacteria. Sci Total Environ: 377:207–213.

Gbadegesin, M.A., Adegoke, A.M., Ewere, E. G. and Odunola, O.A. (2014). Ethanol Extract of Irvingia gabonensis Protects Against Sodium Arsenite Induced Toxicity in Rats. Nigerian Journal of Physiological Sciences Vol. 29. No. 1: 029 – 036.

Harborne, J. B. (1973). Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall, London.

Hill, A. F. (1952). Economic Botany. A textbook of useful plants and plant products. 2nd edn. McGraw-Hill Book Company Inc, New York USA.

Hughes, M. F., Beck, B. D., Chen, Y., Lewis, A. S. and Thomas, D. J. (2011). Arsenic exposure and toxicology: a historical perspective. Toxicological Sciences 123, 305–332.

IARC, (1973). Arsenic and inorganic arsenic compounds. In: Some Inorganic and Organometallic Compounds.

Jimoh, F. O. and Oladiji, A. T. (2005). Preliminary studies on Pilostigma thonningii seed: proximate analysis, medicinal composition and phytochemical screening, African Journal of Biotechnology. Vol. 4, No. 12. pp. 1439-1442.

Karumi, Y., Augustine, A. I., Umar, I. A. (2008). Gastroprotective effects of aqueous extract of Adansonia digitata leaf on ethanol-induced ulceration in rats. J. Biol. Sci. 8: 225-228.

Keay, R. W. J. (1989). Trees of Nigeria. Clarendon Press, Oxford; 137p.

Kittakoop, P., Mahidol, C. and Ruchirawat, S. (2014). "Alkaloids as important Scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation". Cur Top Med Chem. Vol. 14, No. 2. pp. 239-252.

Kunitz, M. (1947). Crystalline soybean trypsin inhibitor: II. General properties. The Journal of General Physiology 20: 291–310.

Mallick, P., Mallick, J. C., Guha, B. and Khuda-Bukhsh, A. R. (2003). Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice. BMC Complement Altern Med 3: 7.

Matter, B. and W. Schmid. (1971). Bone Marrow Toxicity. Mutation Res 12:417-25.

Munro, A. and Bassir, O. (1969). Oxalates in Nigerian vegetables. West African Journal of Biology and Applied Chemistry, 12: 14-18.

Nair, S., Nagar, R. and Gupta, R. (1998). Antioxidant phenolics and flavonoids in common Indian foods. Journal of the Association of Physicians of India 46: 708-71

O'Neil, L., Kim-Standage, W. S., Hughes, G. B. and Murray, K. B. (2000). In: Watson, R. R., 2000, Ed. Vegetables, Fruits and Herbs in Health Promotion, C. R. C. Press, USA.

Odunola, O. A., Akinwumi, K. A., Ogunbiyi, B. and Tugbobo, O. (2007). Interaction and Enhancement of the Toxic Effects of Sodium Arsenite and Lead Acetate in Wistar Rats. African Journal of Biomedical Research 10: 59 – 65.

Preston, R. J., Dean, B. J., Galloway, S., Hoden, H., Mcfee, A.F. and Shelby, M. (1987). Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. Mutat Res. 189: 157-65.

Ramadan, A., Harraz, F. M. and El-Mougy, S. A. (1994). Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of Adansonia digitata. Fitoterapia, 65: 418-422.

Reddy, N., Anjum, M., Aveti, S. and Raju, G. M. (2015). Hepatoprotective Activity of Methanolic Extract of Whole Plant of Rhynchosia beddomei in Wistar Rats. J Pharm Chem Biol Sci: 3(3):378-387

Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. Critical Reviews on Food Science and Nutrition. Vol. 45, No. 4. pp. 287-306.

Sibibe, M., Williams, J. T. (2002). Baobab – Adansonia digitata. Fruits for the future 4, International Centre for Underutilised Crops, Southampton, UK, 96p.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152–178.

Smith, A. H., Goycolea, M., Haque, R. and Biggs, M. (1998). Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. Am J Epidemiol:147:660-9.

Smith, A. H., Lingas, E. and Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ: 78:1093-103.

Sulaiman, L. K., Oladele, O. A., Shittu, I. A., Emikpe, B. O., Oladokun, A. T. and Meseko, C. A. (2011). In-ovo evaluation of the antiviral activity of methanolic root-bark extract of the African Baobab (Adansonia digitata Lin. African Journal of Biotechnology Vol. 10 (20), pp. 4256 – 4258.

Szasz, G. (1974). Determination of GGT activity. Methods of Enzymatic Analysis. 2nd English edition. Academic Press Inc. New York pp 715 – 720.

Tanko, Y., Yerima, M., Mahdi, M. A., Yaro, A. H., Musa, K. Y., Mohammed, A. (2008). Hypoglycemic Activity of the methanolic Stem Bark of Adansonia digitata extract on Blood Glucose Levels of Streptozocin-Induced diabetic Wistar Rats. International Journal of Applied Research in Natural Product. Vol.1(2).pp32-36.

Tietz, N. W., Burtis, C. A., Ducan, P., Ervin, K., Petitclera, C. J., Rinker, A. D., Shney, D. and Zygowicz, E. R. (1983). A reference method for measurement of alkaline phosphatase activity in human serum. Clinical Chemistry 29: 751-761.

Van Buren, J. P. and Robinson, W. B. (1981). Formation of Complexes between Protein and Tannic Acid. Journal of Agric Food Chemistry 17: 772 –777.

Vimalanathan, S., Hudson, J. B. (2009). Multiple inflammatory and antiviral activities in Adansonia digitata (Baobab) leaves, fruits and seeds. J. Med. Plan. Res. 3(8): 576-582.

Yeshua'u, M., Hamza, M. M and Abdullahi, N. (2010). Antibacterial activity of Adansonia digitata stem bark extracts on some clinical bacterial isolates. International Journal of Biomedical and Health Sciences. Vol.6.No.3 129-135.

Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science 8: 203-208.

Harbone, J.B. (1998). Phytochemical Methods Chapman and Hall Ltd London.

Niger. J. Physiol. Sci. 35 (December 2020): 181 - 186
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Haematological Studies and Micronucleus Assay of Straw-Coloured Fruit Bats (*Eidolon helvum*)

Anosike F.[1], Lanipekun D.O.[1], Adebiyi O.F.[2], Ogunsuyi O.M.[3], Bakare A.A.[3] and *Olopade J.O.[1]

[1]*Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria.*
[2]*Department of Veterinary Biochemistry and Physiology, University of Ibadan, Ibadan, Nigeria.*
[3]*Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria.*

Summary: The straw-coloured fruit bats (*Eidolon helvum*) are the most widely distributed megachiropteran species in Africa. Studies have shown that they migrate, and are likely exposed to environmental pollutants across population. This study was designed to investigate genotoxicity via the bone marrow micronucleus assay and haematological alterations of *Eidolon helvum* in the tropics. Healthy straw-coloured fruit bats (*Eidolon helvum; n=20*) were captured from two geographical regions, Ogun and Gombe States in Nigeria and were grouped based on sex and age. Blood samples were collected for haematology and osmotic fragility, and bone marrow samples for genotoxicity studies. Results showed no significant differences in erythrocytes and leucocytes values across age and sex. The erythrocytes osmotic fragility was higher in juvenile than in adults at 0 and 0.1%NaCl, while it was higher in adult males than in adult females at 0 and 0.3% NaCl. The erythrocytes and leucocytes parameters in straw-colored fruit bats were within the reference values seen in literature except the higher monocyte counts suggesting chronic inflammation. There were increased levels of micronucleated polychromatic erythrocytes and normochromatic erythrocytes in the straw-coloured fruit bats indicating genotoxicity and cytotoxicity, respectively. The present study provided baseline research data on the haematology and micronucleus profile of the straw-coloured fruit bats in Nigeria. This is perhaps the first study on haematology and micronucleus assay of in straw-colored fruit bats in the tropics.

Keywords*: Straw-coloured fruit bats, Erythrocyte osmotic fragility, Micronucleus, Haematology*

**Address for correspondence: jkayodeolopade@yahoo.com; Tel:* +234 8023360829

Manuscript Received – August, 2020; Accepted - October, 2020

INTRODUCTION

Bats are mammals belonging to the order Chiroptera, which is the second most diverse among mammalian orders, exhibiting great physiological and ecological diversity (Hutson *et al* ., 2001). Their forelimbs form webbed wings, making them the only mammals naturally capable of true and sustained flight. The straw-colored fruit bat (*Eidolon helvum*) is a large fruit bat that is the most widely distributed of all the African megabats. Three subspecies have been previously described: *Eidolon helvum* dupreanum (from Madagascar), *Eidolon helvum* helvum (from continental Africa) and *Eidolon helvum* sabaeum (from Arabia) (Skinner and Chimimba, 2005). The straw-coloured fruit bat got its name from the yellow fur on their back. Its belly has a brown color while the skin of its wings is dark brown or black.

Blood is a tissue consisting of red blood corpuscles (erythrocytes), white corpuscles (leukocytes), and platelets. It transports oxygen, carbon dioxide, metabolites, products of digestion, hormones, enzymes and clotting factors. Blood profile is important in evaluating the physiological conditions as well as the nutritional status of animal populations (Herdt, 2000). External factors such as season, time of the day, food availability and quality can affect the blood profile of animals. Other intrinsic factors like age, gender, environment and reproductive state also play important roles (Bezerra *et al* ., 2017; Shawaf *et al* ., 2018). These indices have been employed in effectively monitoring the responses of bats to environmental factors/stressors and thus their health status under such adverse conditions (Bandouchova *et al* ., 2018). They can thus provide substantial information once reference values are established under standardized conditions. In the past, authors have described haematological parameters in different species of bats in some geographical region of the world (Wołk and Ruprecht, 1988; Paksuz *et al* ., 2009; Selig *et al* ., 2016; Ekeolu and Adebiyi, 2018). Results from these studies reflected a great variation in the haematological parameters of bats based on breeds, ages, sexes and location. There is however a paucity of physiological reference values for the straw-colored bat; *E. helvum* in the tropical environments.

In vivo micronucleus (MN) assay has been widely used to evaluate the cytogenetic damage induced by environmental xenobiotics, due to its technical simplicity and sensitivity to xenobiotics capable of inducing genotoxic effects on the DNA (MacGregor *et al* ., 1990; Krishna and Hayashi, 2000). It was recommended as a primary test by regulatory agencies for the safe assessment of carcinogenic

and mutagenic chemicals (Morita *et al* ., 1997). Although it was initially developed with mammalian species (Heddle, 1973; Schmid, 1975), it is now extensively applied in the cytogenotoxic assessment of various xenobiotics in lower vertebrates like toads (Malladi *et al* ., 2007), birds (Huang *et al* ., 2007; Alimba and Bakare, 2016) and fish (Cavas, 2008). This is attributed to its reproducibility, cost effectiveness, rapidity and suitability of scoring micronucleated cells at interphase in both laboratory and field studies (Morita *et al* ., 1997).

The purpose of this study is to describe the morphological features of the blood cells as well as haematology baseline values, including erythrocyte osmotic fragility and induction of micronuclei in the bone marrow cells of Eidolon bats Nigeria. This information will assist health monitoring and assessment of this migratory mammal, and as a sentinel animal for environmental pollution.

MATERIALS AND METHODS

Experimental Animals: Twenty straw-colored fruit bats (*Eidolon helvum*; 5 Juveniles and 15 Adults) were used for this study. They were captured with mist nets from Ogun and Gombe State representing the Southwestern and North-eastern zones of Nigeria respectively between May and August 2018. They were immediately removed from the net and put in a metal cage and stabilized for 72 hours; the bats were anesthetized intramuscularly using ketamine at 80-90mg/kg body weight.

Haematological Studies: Blood samples were collected from the uropatagial vein into bottles containing EDTA (2mg/mL) as anticoagulant. The full blood count was analyzed using automated hematology analyzer. Fresh smear of adult bats was fixed with 99.9% methanol and stained with Giemsa stain for blood cell morphology.

Osmotic Fragility Test (OFT): Erythrocytes osmotic fragility was determined according to the method described by Oyewale (1992). 0.02 mL of blood was added to tubes containing increasing concentrations of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.3, 0.5, 0.7, 0.9% NaCl concentration). The tubes were gently mixed and incubated at room temperature (29°C) for 30 min. The content of each tube was centrifuged at 686g for 10 min, and the supernatant decanted for measurement. Optical density of the supernatant was determined at 540nm using a digital spectrophotometer (model UV-1650; Shimadzu, Tokyo, Japan). Haemolysis in each tube was expressed as a percentage, while taking haemolysis in distilled water (0% NaCl) as 100%.

Micronucleus Assay: The bone marrow cells preparation for the MN assay was carried out according to Schmid (1975) with slight modifications. Briefly, the straw-colored fruit bats (*n*=5) were and both femurs of the bats were excised and the bone marrow cells were flushed into Eppendorf tubes with 1 mL of Foetal Bovine Serum (FBS) (Sigma Aldrich, Germany). The micropipette was used to carefully dislodge the bone marrow cells, and centrifuged at 380 g for 5 min. This procedure was repeated three times to properly remove fatty tissues. After discarding the supernatant, fresh FBS (300 µL) was added to the pellets and the cells carefully mixed. Five thin smears were prepared for each bat, stained with May-Grunwald and Giemsa stains. For each bat, 1000 polychromatic erythrocytes with micronucleus were scored at X1000 using the light microscope (Micromaster, Fisher Scientific, China). The percentage PCE: NCE was used as the cytotoxicity index (Krishna and Hiyashi, 2000).

Ethical approval for this work was obtained from University of Ibadan Ethics Committee (UI-ACUREC/App/2016/015)

Statistical Analysis

Data obtained were analyzed with Student's t-test and expressed as Mean ± SD using GraphPad Prism 7. The results were considered statistically significant for P values ≤ 0.05.

RESULTS

Complete blood count: The haematological parameters of straw-colored fruit bats based on age and sex are presented in Tables 1 and 2. The number of red blood cells (RBC) count, packed cell volume (PCV), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not statistically ($p>0.05$) different between different ages and sex in the bats. In the adult male and female, the RBC fell below the reference values documented in the temperate by Selig *et al* . (2016) whereas in the juvenile; both RBC and Hb values fell below these reference values. The neutrophil count was significantly lower in juvenile (1.3 ± 1.07) compared with adults (2.13 ± 1.55) (Table 3). Other leucocytes parameters; WBC, neutrophils, lymphocytes, monocytes and eosinophils of adults were not significantly different ($p >0.05$) in the adults and juvenile. The neutrophils values reported were lower than the reference value by Selig *et al* . (2016). The monocytes count across ages and sexes (Table 4) observed in the present study were higher than the reference values.

Table 1:
Erythrocytes Values of Adult and Juvenile Straw-coloured Fruit Bats in Nigeria

PARAMETERS	ADULT (n=15)	JUVENILE (n=5)	REFERENCE VALUES (Selig *et al ., 2016*)
RBC ($x10^6$ mm)	7.65 ± 1.60	7.42 ± 0.81	7.74-10.89 (9.47± 0.69)
PCV (%)	39.50 ± 8.34	38.76 ± 4.19	33.30-51.00 (42.68± 19.3)
Hb(g/dl)	12.33 ± 2.30	11.60 ± 1.36	12.00-16.00 (14.89± 1.03)
MCV (fL)	51.67 ± 4.88	45.10 ± 17.15	40.10-53.90 (45.08± 2.97)
MCH (pg)	16.37 ± 1.31	18.62 ± 6.59	14.20-17.30 (15.73± 0.73)
MCHC (g/dl)	31.51 ± 2.59	34.18 ± 9.83	30.00-37.20 (34.99± 1.94)

Table 2:
Erythrocytes Values of Adult Straw-Coloured Fruit bats in Nigeria

PARAMETERS	MALES (n=7)	FEMALES (n=8)	REFERENCE VALUES (Selig *et al ., 2016*)
RBC ($x10^6$ mm^3)	7.70 ± 1.59	7.60 ± 1.70	7.74-10.89 (9.47± 0.69)
PCV (%)	41.09 ± 8.97	38.11 ± 8.08	33.30 – 51.00 (42.68± 19.3)
Hb (g/dl)	12.47 ± 2.31	12.20 ± 2.44	12.00 -16.00 (14.89± 1.03)
MCV (fL)	53.29 ± 5.22	50.25 ± 4.40	40.10 -53.90 (45.08± 2.97)
MCH (pg)	16.63 ± 1.04	16.15 ± 1.55	14.20 -17.30 (15.73± 0.73)
MCHC (g/dl)	30.79 ± 3.23	32.14 ± 1.85	30.00 -37.20 (34.99± 1.94)

Table 3:
Leucocytes Values of Adult and Juvenile Straw-Coloured Fruit Bats in Nigeria

PARAMETERS	ADULT (n=15)	JUVENILE (n=5)	REFERENCE VALUES (Selig *et al .*, 2016)
WBC $x10^9$ L	4.78 ± 2.42	4.50 ± 2.64	1.20 -7.30 (3.19± 1.48)
Neutrophils ($x10^9$ L)	2.13 ± 1.55 (44.56 ± 22.00)	1.30 ± 1.07 (28.89 ± 25.33)	0.29-6.00 (1.36± 1.32)
Lymphocytes ($x10^9$ L)	2.01 ± 1.48 (42.05 ± 22.60)	2.48 ± 2.89 (55.11 ± 26.68)	0.64-4.00 (1.70± 0.80)
Monocytes ($x10^9$ L)	0.43 ± 0.32 (9.00 ± 3.83)	0.44 ± 0.17 (10.47 ± 4.55)	0.00 -0.30 (0.05± 0.07)
Eosinophils ($x10^9$ L)	0.21 ± 0.14 (4.39 ± 1.64)	0.28 ± 0.11 (6.22± 3.51)	0.00 -0.89 (0.07± 0.18)

Table 4:
Leucocytes Values of Adult Straw-Coloured Fruit bats in Nigeria

PARAMETERS	MALES (n=7)	FEMALES (n=8)	REFERENCE VALUES (Selig *et al ., 2016*)
WBC $x10^9$ L	3.90 ± 1.42	5.6 ± 2.90	1.20 -7.30 (3.19± 1.48)
Neutrophils ($x10^9$ L)	1.51 ± 0.76 (38.72 ± 25.50)	2.71 ± 1.87 (48.40 ± 19.99)	2.90 -6.00 (1.36± 1.32)
Lymphocytes ($x10^9$ L)	1.79 ± 1.42 (45.89 ± 27.40)	2.22 ± 1.60 (39.64 ± 19.46)	0.64-4.00 (1.70± 0.80)
Monocytes ($x10^9$ L)	0.40 ± 0.37 (10.26 ± 5.18)	0.46 ± 0.29 (8.21 ± 2.49)	0.00 -0.30 (0.05± 0.07)
Eosinophils ($x10^9$ L)	0.2 ± 0.16 (5.13 ± 2.19)	0.21 ± 0.14 (3.75 ± 0.99)	0.00 -0.89 (0.07± 0.18)

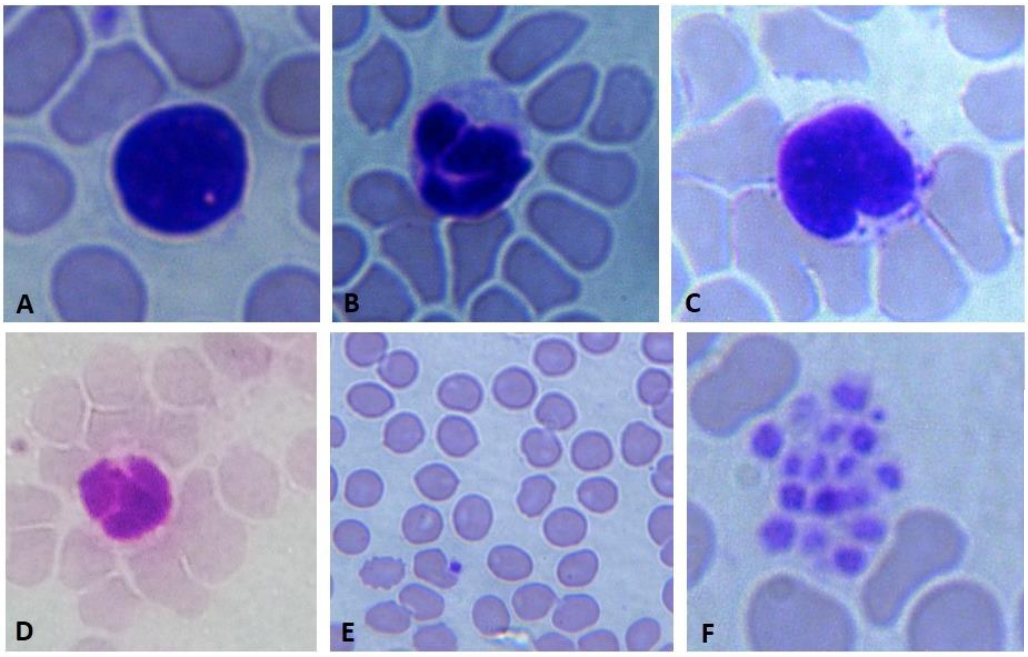


Plate 1:
Photomicrograph of blood smear showing blood cells of the Adult Straw-coloured fruit bat A-F. Lymphocyte (A); Neutrophil (B); Monocyte (C); Eosinophil (D); Erythrocytes (E); Platelets (F).

Blood Smear: There were no morphological differences in the blood smear across ages and sexes of the straw-colored fruit bats (Plate 1A-F). Lymphocytes were the most abundant leukocytes in the blood smears (Plate 1A). They were round or slightly elliptical, contained small amounts of cytoplasm, and were of two different sizes large lymphocytes and of small lymphocytes. Neutrophils, the second most abundant leukocytes, were similar in size to large lymphocytes but easy to distinguish by the size and shape of the nucleus (Plate 1B). The nucleus of monocytes was U - or bean-shaped, and more than two-thirds of the cell size (Plate 1C). Eosinophils, the fourth most abundant leukocytes (Figure 1D), were also spherical in shape, had some visible large, granules in their cytoplasm. Erythrocytes were biconcave with no nucleus (Plate 1E). Platelets were seen as fragments (Plate 1F) in the blood smear.

Erythrocyte Osmotic Fragility: The erythrocyte osmotic fragility was higher in juvenile than in adults at 0 and 0.1% NaCl (Figure 1). On the other hand, erythrocyte osmotic fragility was higher in adult males than in adult females at 0 and 0.3% NaCl (Figure 2). However, there were no statistically significant ($p > 0.05$) differences in the above results.

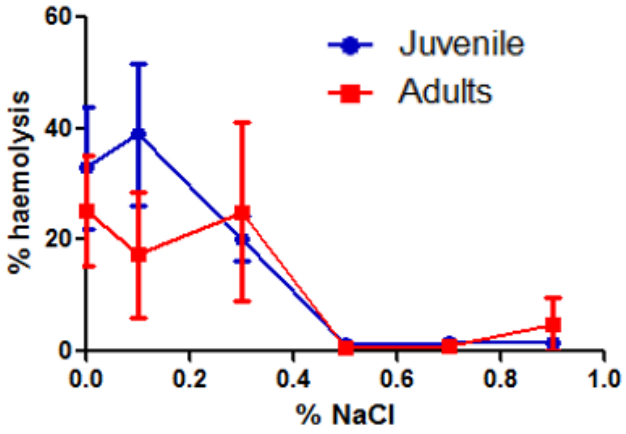


Figure 1:
Showing erythrocyte osmotic fragility test in juvenile and adults Straw-coloured fruit bat

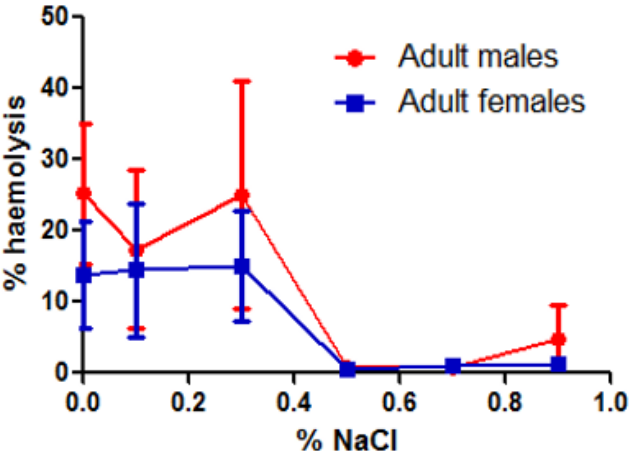


Figure 2:
Showing erythrocyte osmotic fragility test in adult males and females Straw-coloured fruit bat

Micronucleus Assay: This assay as a biomarker for genotoxicity indicated an elevated level of micronucleated polychromatic erythrocytes (MNPCE) in all the straw-coloured fruit bats (Plate 2). The PCE/NCE an index for assessing cytotoxicity indicates an abnormal proliferation from polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) (Table 5).

DISCUSSION

The erythrocytes and leucocytes parameters in straw colored fruit bats were within the reference range as reported by Selig *et al* . (2016) except for the monocytes which were higher. High monocytes count is an indication of chronic inflammation, but this may be a normal phenomenon. It has been reported that the average percentage of monocytes is high in all species of bats (Riedesel, 1977).

The RBC count 7.54 $x10^{12}$/L is lower than 9.15 $x10^{12}$/L reported in *Epomops franqueti* (Ekeolu and Adebiyi, 2018), 12.39 $x10^{12}$/L in the serotine bat (Wolk and Ruprecht, 1988) and 11.35 $x10^{12}$/L in *Myotis myotis* (Albayrak *et al* ., 2016). There was no significant difference in the WBC counts of adults when compared with Juvenile likewise in adult males when compared with adult females in this study. The total WBC counts 4.69 $x10^{9}$/L obtained in this study is similar to that reported in the insectivorous microchiropterans of the temperate breeds, *Myotis myotis* with 4.87 $x10^{9}$ /L (Albayrak *et al* ., 2016) but higher than some other species such as vampire bats, 3.68$x10^{9}$/L (Arevalo *et al* ., 1992), *Myotis veliper*, 2.20 $x10^{9}$/L (Krutzsch and Hughes, 1959) and *Myotis elegans*, 2.05 $x10^{9}$/L. However, they differ from 13.46 $x10^{9}$/L obtained for franquet's fruits bat as reported by Ekeolu and Adebiyi (2018). Blood cells morphology is similar to that reported in the little brown bat by Cooper *et al* . (2014) and in some species of insectivorous bats by Paksuz *et al* . (2009). This study is the first to our knowledge to provide baseline information on the haematology and morphology in the straw-colored fruit bats in the tropics.

Osmotic fragility is a measure of the resistance of RBC to lysis as a function of increasing NaCl concentration. Females of the *Epomops franqueti* have higher erythrocytes osmotic fragility than the males of the same species (Ekeolu and Adebiyi, 2018). Although the erythrocyte osmotic fragility was higher in the juvenile than in the adults, there was no significant difference. It shows that the adult of *Eidolon* bats are more resistant to intravascular hemolysis. Similarly, erythrocytes osmotic fragility is higher in the adult males than in adult females. It indicates that the adult males are more fragile and less resistant to lysis.

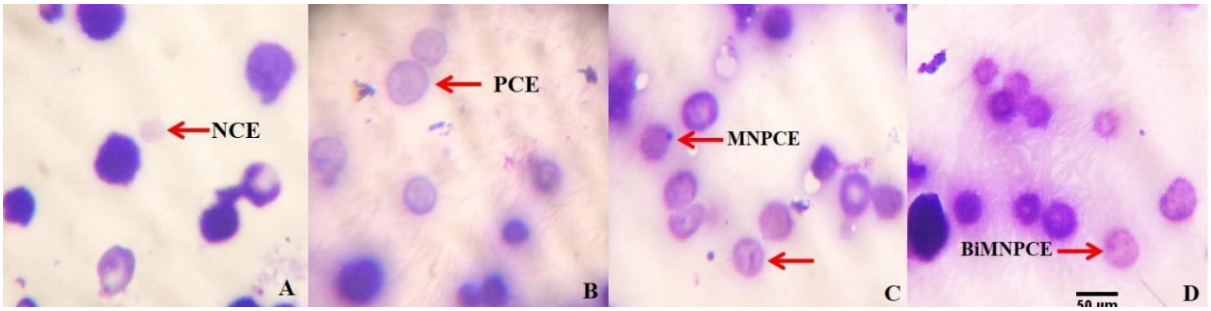


Plate 2:
Showing bone marrow smear of straw-colored fruit bat for micronucleus assay
A) NCE: Normochromatic erythrocyte; B) PCE: Polychromatic erythrocyte; C) MNPCE: Micronucleated erythrocyte; D) BiMNPCE: Bi-nucleated polychromatic erythrocyte. x1000 magnification.

Micronucleus used as a biomarker for genotoxicity in this study indicated that there may be environmental toxins (heavy metals, organic and inorganic chemicals), which accumulated over time in the bats. Since they are migratory birds, there is a great likelihood that the environment is highly polluted with genotoxins that are capable of inducing DNA damage, hence, increasing the micronucleated polychromatic erythrocytes. Naidoo *et al* . (2014) reported that pollutant-exposed bats have significantly higher DNA damage and diminished antioxidant capacity. In the study, there was an elevated level of MNPCE which is indicative that the environmental toxicants are likely clastogens and or aneugens inducing chromosome/chromatid breakage and inhibiting spindle fibres, respectively. In addition, it was observed that there was an abnormal rapid rate of proliferation from PCE to NCE, which is indicative of bone marrow toxicity and aging (Gonzalez-Borroto *et al* ., 2003; Heuser *et al* ., 2008) and likely reduction of the life span of the bats.

In conclusion, with the knowledge that bats are known reservoirs of many infectious disease that are transmissible to man and other mammals, the result of this study may provide a good basis to further explain the role of WBC and the immune system of this bat species following inflammation or infections with a means to early detection and control strategies for disease outbreak. The mechanism of the DNA damage induced by these environmental toxicants is unknown; however, further studies require identification of these toxicants as well as their mechanistic pathway in the induction of DNA damage. This is of importance to the general public as these toxicants may affect human health and the environment over time.

Acknowledgement

The authors acknowledge the Alexander von Humboldt Research Group Linkage Grant that was used for the bench work of this project.

REFERENCES

Albayrak, I., Özcan, H.B and Baydemir, M. (2016). Some hematological parameters in Myotis myotis and Myotis blythii (Mammalia: Chiroptera) in Turkey. Turk. J. Zool., 40,388–91.

Alimba, C.G. and Bakare, A.A.(2016). In vivo micronucleus test in the assessment of cytogenotoxicity of landfill leachates in three animal models from various ecological habitats. Ecotoxicology,25, 310–319.

Arevalo, F., Parez, S.G and Lopez, L.P. (1992). Seasonal changes in blood parameters in the bat species Rhinolophus ferrumequinum and Miniopterus schreibersii. Arch. Physiol.Biochem.,100, 385–7.

Bandouchova, H., Bartonička, T., Berkova, H., Brichta, J., Kokurewicz, T., Kovacova, V., Linhart, P., Piacek, V., Pikula, J., Zahradníková, A. and Zukal, J.(2018). Alterations in the health of hibernating bats under pathogen pressure. Sci. Rep., 8,461-465.

Bezerra, L.R., Oliveira, W.D., Silva, T.P., Torreão, J.N., Marques, C.A., Araújo, M.J. and Oliveira, R.L. (2017). Comparative hematological analysis of Morada Nova and Santa Inês ewes in all reproductive stages. Pesquisa Veterinária Brasileira, 37, 408 – 414.

Cavas. T. (2008). In vivo genotoxicity of mercury chloride and lead acetate: micronucleus test on acridine orange stained fish cells. Food Chem. Toxicol., 46, 352–358.

Cooper, M., Hooper, S., Amelon, S. and Wiedeneyer C. (2014). Haematological and Electrolyte changes in little brown bats treated for white nose syndrome. In: merial-NIH Veterinary scholars' symposium.

Ekeolu, O.K. andAdebiyi, O.E. (2018). Hematology and erythrocyte osmotic fragility of the Franquet's fruit bat (Epomops franqueti). J Basic Clin Physiol Pharmacol. doi.org/10.1515/jbcpp-2017-0169.

Gonzalez-Borroto, J.I., Creus, A., Marcos, R., Molla, R., Zapatero, J. (2003). The mutagenic potential of the furylethylene derivative 2-furyl-1-nitroethene in the mouse bone marrow micronucleus test. Toxicol. Sci.,72, 359-362. 10.1093/toxsci/kfg038.

Heddle, J.A. (1973). A rapid in vivo test for chromosome damage. Mutat. Res., 18, 187–192.

Herdt, T.H. (2000). Variability Characteristics and Test Selection in Herdlevel Nutritional and Metabolic Profile Testing. Vet. Clin. N. Am.: Food AnimPract., 16, 387–403. doi:10.1016/s0749-0720(15)30111-0.

Heuser, V., Deandrade, V., Peres, A., Gomesdemacedobraga, L., and Bogochies, J. (2008). Influence of age and sex on the spontaneous DNA damage detected by Micronucleus test and Comet assay in mice peripheral blood cells. Cell Biol. Int., 32, 1223–1229. doi:10.1016/j.cellbi.2008.07.005.

Huang, D., Zhang, Y., Wang, Y., Xie, Z., Ji, W. (2007). Assessment of the genotoxicity in toad Bufo raddei exposed to petrochemical contaminants in Lanzhou Region, China. Mutat. Res., 629, 81–88.

Hutson, A.M., Mickleburgh, S.P. and Racey, P.A. (2001). Microchiropteran Bats: Global Status Survey and Conservation Action Plan, IUCN/SSC chiroptera specialist group, IUCN, Gland, Switzerland.

Krishna, G. and Hayashi, M. (2000). In vivo rodent micronucleus assay; protocol, conduct and data interpretation. Mutat. Res., 20, 155-66.

Krutzsch, P.H. and Hughes, A.H. (1959). Hematological changes with torpor in the bat. J. Mammal, 40,547–54.

MacGregor, J.T., Wehr, C., Henika, P.R., Shelby, M.D. (1990). The in vivo erythrocyte micronucleus test: measurement at steady state increases assay efficiency and permits integration with toxicity studies. Fund. Appl. Toxicol., 14, 513–522.

Malladi, S.M., Bhilwade, H.N., Khan, M.Z, Chaubey, R.C. (2007). Gamma ray induced genetic changes in different organs of chick embryo using peripheral blood micronucleus test and comet assay. Mutat. Res., 630, 20–27.

Morita, T., Asano, N., Awogi, T., Sasaki, Y.F., Sato, S., Shimada, H., Sutou, S., Suzuli, T., Wakata, A., Sofuni, T., Hayashi, M. (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Group 1. 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS MMS. Mutat. Res.,389, 3–122.

Naidoo, S., Vosloo, D. and Corrie-Schoeman, M. (2014). Haematological and genotoxic responses in an urban adapter, banana bat, foraging at waste water treatment works. Eco. Env. Saf., 114, 304-311. doi.org/10.1016/j.ecoenv.2014.04.043.

Oyewale, J.O. (1992). Effects of temperature and pH on osmotic fragility of erythrocytes of the domestic fowl (Gallus domesticus) and guinea fowl (Numida maleagridis). Res. Vet. Sci.,52, 1-4.

Paksuz, S., Paksuz, E.P. and Ozkan, B. (2009). White blood cell (WBC) count of different bat (Chiroptera) species. Trakya. Univ. J. Sci., 10,55-59.

Riedesel, M.L. (1977). Blood physiology. In: "Biology of Bats". Ed. Wimsatt WA. Vol. III. Academic Press: pp485—517, New York.

Schmid, W. (1975). The micronucleus test. Mutat. Res.,31, 9-15.

Selig, M., Lewandowski, A. and Kent, M.S. (2016). Establishment of reference intervals for hematology and biochemistry analytes in a captive colony of straw-colored fruit bats (*Eidolon helvum*). J. Zoo. Wild. Med., 47, 106–112.

Shawaf, T., Hussen, J., Al-Zoubi, M., Hamaash, H., and Al-Busadah, K. (2018). Impact of season, age and gender on some clinical, haematological and serum parameters in Shetland ponies in east province, Saudi Arabia. Int. J. Vet. Sci. Med., 6, 61–64. https://doi.org/10.1016/j.ijvsm.2018.03.007

Skinner, J.D. and Chimimba, C.T. (2005). The Mammals of the Southern African Sub-region. 3rd edition. Cambridge University Press, Cambridge, UK.

Wołk, E. and Ruprecht, A.L. (1988). Haematological values in the serotine Bat, Eptesicus serotinus (Schreber, 1774). Acta Theriol., 33, 545–53.

Niger. J. Physiol. Sci. 35 (December 2020): 187 - 190
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Effect of Tamsulosin Administration on Oral Glucose Tolerance (OGT) In Normal Wistar Rats

Dikko M.[1], Sarkingobir Y.[2] and Umar A.I.[3]

[1]*Department of Pharmacy, Sultan Abdurrahman College of Health Technology, Gwadabawa, Nigeria*
[2]*Department of Biology Shehu Shagari College of Education. Sokoto, Nigeria*
[3]*Department of Biochemistry, Sokoto State University, Sokoto,, Nigeria*

Summary: The objective of this study was to determine the effect of administration of tamsulosin on oral glucose tolerance in normal Wistar rats. Forty (40) male albino Wistar rats were selected and divided into four (4) groups of ten (10) rats each, viz, GROUP I, II, III and IV. Group I (Normal control): Distilled water (5ml/kg), Group II (Positive control): Carvedilol(800µg/kg), Group III (Tamsulosin treated): Tamsulosin (12µg/kg), Group IV (Tamsulosin treated): Tamsulosin (40µg/kg).Different treatments of Distilled water, Carvedilol and Tamsulosin were administered once every day orally for the period of six (6) weeks. After the 6th week of the study, all the treatments were withdrawn for a further 2 weeks (7th and 8th weeks). The Animals underwent 8 hours fasting. OGTT was done at baseline (0th), and then at3rd, 6th, 7thand 8th weeks. The blood glucose of all the animals was measured via tip tail incision at 0hours (pre-glucose load). Then, 2g/kg of D(+)-glucose powder dissolved in distilled water was administered to all the animals orally; after which blood samples were measured via tail tip incision at 30, 60 and 120 minutes using standard glucometer. ANOVA and Tukey Kramer post hoc test was used. The results were revealed therein. At the baseline of the study, 2nd, 3rd week, the groups of rats treated with carvedilol (positive control),tamsulosin high dose (40µg/kg) or low dose (12µg/kg) did not show any significant difference ($P>0.05$) in total area under the oral glucose tolerance curve compared to the normal control group and other inter group comparison. At the 6th week of the study, the group of rats treated with carvedilol (positive control), tamsulosin low dose (12µg/kg) and tamsulosin high dose (40µg/kg) revealed significantly higher values ($P<0.05$) of total area under the oral glucose tolerance curve compared to the normal control group. Other inter-group comparisons were not significantly different ($P>0.05$). At the 7th week of the study, the group of rats treated with carvedilol (positive control), tamsulosin low dose (12µg/kg) and tamsulosin high dose (40µg/kg) revealed no significant differences ($P>0.05$) in total area under the oral glucose tolerance curve compared to normal control group and other inter-groups comparison. At the 8th week of the study (two weeks after treatments withdrawal), only group of rats treated with carvedilol (positive control) revealed significantly higher values ($P<0.05$) of total area under the oral glucose tolerance curve than the normal control group. Other inter-group comparisons were not significantly different. The current study revealed that tamsulosin affects the glucose tolerance of the Wistar rats, thereby causing hyperglycemia.

Keywords*: Tamsulosin, hyperglycemia, Oral glucose tolerance test (OGTT, benign prostatic hyperplasia (BP), carvedilol*

**Address for correspondence: superoxidedismutase594@gmail.com; Tel: +234-9096266980*

Manuscript received: August 2019; Revised version accepted: July, 2020

INTRODUCTION

Glucose tolerance impairment is a state of increased risk of progressing to diabetes. It also increases the risk of cardiovascular disease. Thus, it is a risk factor for future diabetes or adverse outcomes (WHO, 2006). The prevalence of impaired glucose tolerance is increasing among the older men across the various continents of the world (WHO, 2006). Presently, oral glucose tolerance test (OGTT) is regarded as the gold standard for the diagnosis of diabetes (Patrick, 2012). OGTT can be affected by many factors such as carbohydrate intake and duration of fasting before the test, the time of the day the test is carried out, carbohydrate intake during test, activity during the test, caffeine consumption or smoking during the fasting. Therefore Wistar rats are the good candidates to manage and monitor larger samples (Patrick, 2012).

Clinically, hyperglycemia is a persistent blood glucose level above 11mmol/l for more than two (2) hours after meals or over 6.9mmol/l after fasting. Hyperglycemia occurs when insulin is unable to stimulate blood glucose uptake in target tissues mainly skeletal muscles. Medical investigations to establish the presence of hyperglycemia includes oral glucose tolerance test (OGTT) which is used to determine how swiftly glucose is cleared from the blood after its ingestion through oral route (Suresha *et al*., 2013). OGTT is a medical investigation primarily adopted to detect glucose intolerance since it shows post-prandial glucose excursion. OGTT is used to test for diabetes, insulin resistance and the inability of the pancreas beta cells to secrete insulin (Dikko, 2019). OGT is the common glucose tolerance test that is widely used to check how body shuttle glucose from blood into the tissues (United States Library of Medicine, 2020). It is indicative when other tests are insufficient in establishing or ruling out the diabetes diagnosis (Jerry, 2018). That is why it was carried out to determine the ability of tamsulosin to cause hyperglycemia in rats.

Adverse drug reactions (ADRs) refers to any undesirable response presented due to a drug by biological system (more especially humans) at normal doses (WHO, 2005; Umar *et al*., 2010; Umar *et al*., 2016). Due to ADRs many drugs were withdrawn from the market during the last century (Preissner *et al*., 2015). Hyperglycemic effect was the adverse drug reaction (ADR) of tamsulosin in benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) human patients as reported by some past studies (Dikko *et al*., 2020a; Dikko *et al*.,2020b). ADR is one of the leading causes of iatrogenic diseases throughout the world. Globally, it cost about 4 billion USD every year, and leads to about 5% hospital admissions, 28% emergency department's visits, and 5% hospital deaths (Umar *et al*., 2016; Oduala *et al*., 2018). ADRs can cause patients to lose confidence in healthcare or drugs; and on the other hand increase self-medication or precipitate further ADRs. Moreover, cost of ADRs management can be high or precipitate other ADRs, and in turn leading to large burden on the patients, healthcare system, and government (Dikko *et al*., 2020a; Dikko *et al*., 2020b). One of the escalating factors of the trend is the underreporting of ADR from the patients and healthcare givers. Sources that are utilized to detect unidentified ADRs of drugs include anecdotal reports, observational studies, clinical trials, systematic reviews, or animal data (Onakpoya *et al*., 2016). Certain measures are followed to minimize ADRs such as careful medication review, good education to patients and healthcaregivers, monitoring and pharmacovigilance among others (Woodcock, 2016; Chika *et al*., 2018; Ganiyu and Erah, 2018). The objective of this study was to determine the effect of administration of tamsulosin on oral glucose tolerance in normal Wistar rats.

MATERIALS AND METHODS

Animals: Seventy (70) male adult albino Wistar rats were obtained from the breeding units of Faculty of Veterinary Sciences of University of Ilorin, Nigeria. The rats were kept in the animal house of the Department of Pharmacology and Therapeutics, Usmanu Danfodio University Sokoto in plastic cages with bottoms (freshly spread with a wood saw to absorb urine) at room temperature with 12 hours light/12 hours dark cycle. Cages were cleaned daily and disinfected weekly with 70% alcohol. The rats were left for fourteen (14) days acclimatization. Tap water and grower feeds pellets product were supplied ad libitum.

Experimental Design: Forty (40) male albino Wistar rats were selected from the 70 rats purchased, using random number generator (computer software) and divided into four (4) groups of ten (10) rats each, viz, GROUP I, II, III and IV. They were allowed for three (3) days before the commencement of the study.

- Group I (Normal control): Distilled water(5ml/kg)
- Group II (Positive control): Carvedilol(800µg/kg)
- Group III (Tamsulosin treated): Tamsulosin (12µg/kg)
- Group IV (Tamsulosin treated): Tamsulosin(40µg/kg)

Different treatments of Distilled water, Carvedilol and Tamsulosin were administered once every day (during the research course) through oral route using metal cannula attached to a 2ml syringe for the period of six (6) weeks. After the 6th week of the study, all the treatments were withdrawn for a further 2 weeks (7th and 8th weeks). During the withdrawal period, only water and food were served ad libitum (Suresha *et al*., 2013; Chika *et al*., 2018).

Oral glucose tolerance test (OGTT) in normal rats administered with tamsulosin

OGTT was done at baseline (0th), and then at3rd, 6th, 7thand 8th weeks. Prior to each OGTT, animals underwent 8 hours of fasting. The blood glucose of all the animals was measured via tip tail incision at 0hours (pre-glucose load). Then, 2g/kg of D(+)-glucose powder dissolved in distilled water was administered to all the animals orally (Suresha *et al*., 2013); after which blood samples were measured via tail tip incision at 30, 60 and 120 minutes. A Standardized digital glucometer (Accu check) was used to measure blood glucose levels. Glucometer standardization was done by testing rat plasma with standard (glucose oxidase) and glucometer methods in order to assess their consistency (Suresha *et al*., 2013; Chika *et al*., 2018). ANOVA was used then followed by Tukey Kramer post hoc test.

RESULTS

Oral glucose tolerance test at baseline (0th) of the study in normal rats administered with tamsulosin: At the baseline of the study, the groups of rats treated with carvedilol (positive control),tamsulosin high dose (40µg/kg) or low dose (12µg/kg) failed to show any significant difference ($P>0.05$) in total area under the oral glucose tolerance curve (Fig. 1A) compared to the normal control group and other inter group comparison.

Oral glucose tolerance test at 3rd week of the study in normal rats administered with tamsulosin: At the 3rd week of the study, the groups of rats treated with carvedilol (positive control),tamsulosin high dose (40µg/kg) or low dose (12µg/kg) did not show any significant difference ($P>0.05$) in total area under the oral glucose tolerance curve (Fig 1B) compared to the normal control group and other inter group comparison.

Oral glucose tolerance at 6th week of the study in normal rats administered with tamsulosin: At the 6th week of the study, the group of rats treated with carvedilol (positive control), tamsulosin low dose (12µg/kg) and tamsulosin high dose (40µg/kg) revealed significantly higher values ($P<0.05$) of total area under the oral glucose tolerance curve (Fig. 1C) compared to the normal control group. Other inter-group comparisons were not significantly different ($P>0.05$).

Oral glucose tolerance test at 7th week (one week after tamsulosin withdrawal) of the study in normal rats administered with tamsulosin: At the 7thweek of the study, the group of rats treated with carvedilol(positive control),tamsulosin low dose (12µg/kg)and tamsulosin high dose (40µg/kg)revealed no significant differences ($P>0.05$)in total area under the oral glucose tolerance curve (Fig 1D) compared to normal control group and other inter-groups comparison.

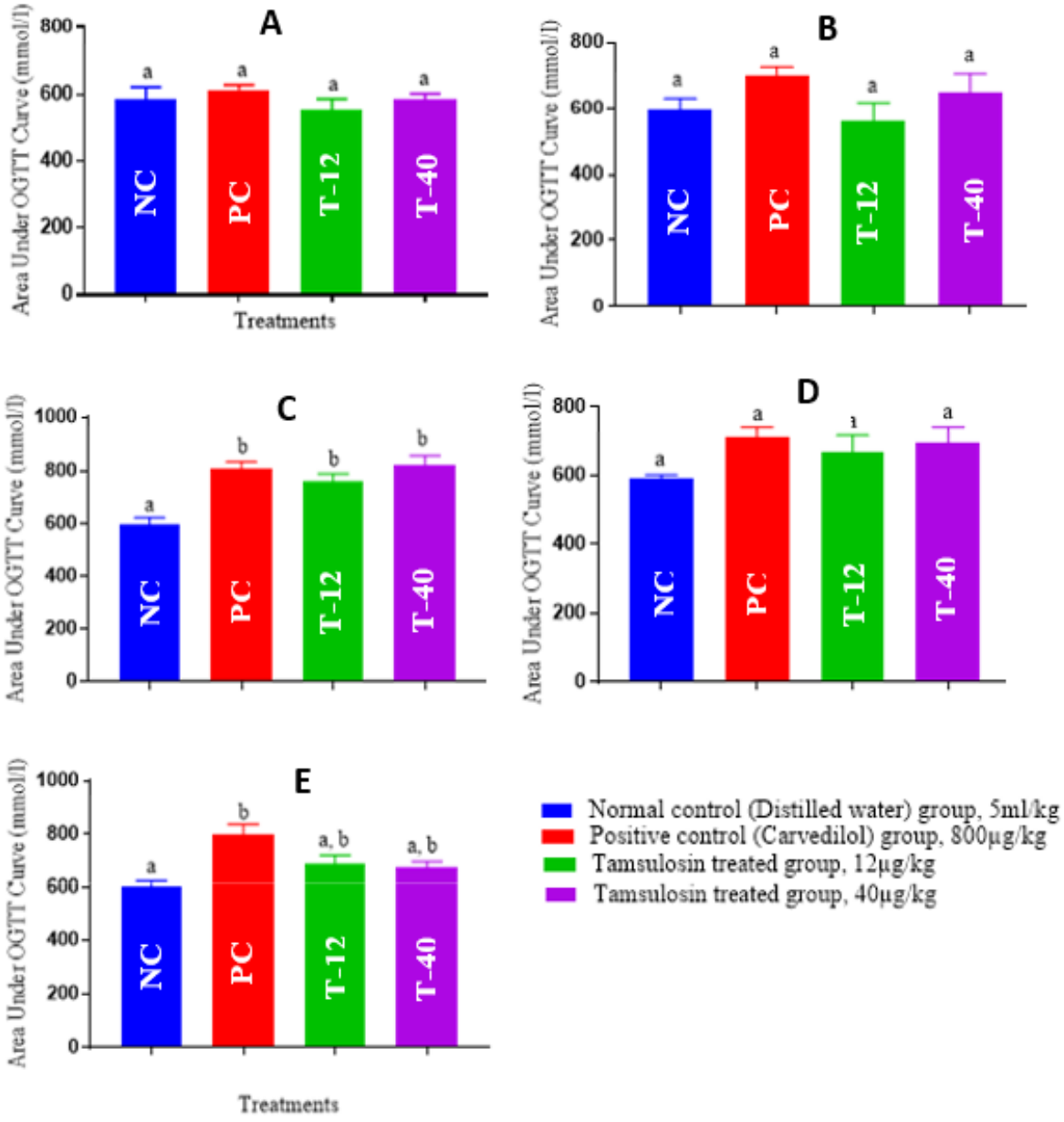


Figure 1:
Effect of tamsulosin on the total area under the oral glucose tolerance curve at baseline or week 0 (A), week 3 (B), week 6 (C), week 7 (D) and week 8 (two weeks after tamsulosin withdrawal) of the study.
Each bar represents Mean ± SEM (n=5). Groups with different lower-case letters are significantly different.
NC – Normal control; PC – Positive control; T-12 – 12µg/kg Tamsulosin treated animals; T-40 – 40µg/kg Tamsulosin treated animals

Oral glucose tolerance test at 8th week (two weeks after tamsulosin withdrawal) of the study in normal rats administered with tamsulosin: At the 8th week of the study (two weeks after treatments withdrawal), only group of rats treated with carvedilol (positive control) revealed a significantly higher values ($P<0.05$) of total area under the oral glucose tolerance curve than the normal control group. Other inter-group comparisons were not significantly different ($P>0.05$; Fig. 1E).

DISCUSSION

The objective of this study was to determine the effect of administration of tamsulosin on glucose tolerance in normal Wistar rats. This study shows that carvedilol and tamsulosin affect glucose tolerance by inducing hyperglycemia in normal rats. Some reports said that tamsulosin cause an impaired oral glucose tolerance and elevated fasting blood glucose in normal rats due to its ability to block L-type voltage gated calcium ion channels (Suresha *et al*., 2013). Calcium ion channels of pancreatic beta-cells are involved in the regulation of insulin secretion (Satin, 2000). Blockage of calcium channel ions by carvedilol might inhibit the influx of intracellular calcium ions thereby impairing insulin secretion, leading to the development of hyperglycemia (Jacob *et al*., 1996). Dikko *et al* (2020) reported that tamsulosin administration in BPH/ LUTS patients lead to impairment of oral glucose tolerance thereby causing hyperglycemic effect.

Moreover, several mechanisms might be the brain behind the observed ability of tamsulosin to cause impairment of glucose tolerance and elevated blood glucose in Wistar rats. It might be due to the reported effect of tamsulosin in blocking alpha-1 adrenoceptors in

experimental animals (Shivaprasad *et al*., 2015). Several studies have reported the important role played by alpha-1adrenoceptors in blood glucose homeostasis in experimental animals and humans through the regulation of blood glucose uptake. Parable, Cheng *et al*. (2000) cited an improvement in glucose uptake into isolated white adipocytes when methoxamine (an alpha 1 agonist) was administered to Wistar rats to stir alpha-1 adrenoceptors; Boschmann *et al*., (2002) and Flechtner-Mors *et al*., (2004) reported some clinical studies revealing that stimulation of alpha-1 adrenoceptors by phenylephrine (an alpha-1agonist) and noradrenaline (non-selective alpha agonist) cause an increase in glucose uptake in human. On the other hand, it has been documented that blockade of alpha 1 receptors leads to impairment in the tissue uptake of blood glucose both invitro and invivo (Cheng *et al*., 2000; Shivaprasad *et al*., 2015). Another possible mechanism for such effects is through increasing insulin secretion. A previous study showed an elevation in plasma insulinlevel (hyperinsulinemia) when alpha-1 adrenoceptors were blocked in experimental rats and humans (Ahrén *et al*., 2008). And this negative effect on glucose tolerance of the rats (which have similar biology with humans) is an ADR that can diminish the enthuastic use of tamsulosin. And it serves as a threat, because hyperglycemia is a factor that goes hand in hand with glucose intolerance, beta-cells inefficiency, and other metabolic syndrome problems threatening public health (Umar etal., 2010; Bilbis etal.,2012).

In conclusion, the current study revealed that tamsulosin affects the glucose tolerance of the Wistar rats, thereby causing hyperglycemia..

REFERENCES

Ahrén, B., Lundquist, I., and Järhult, J. (2008). Effects of α1-, α2- and β-adrenoceptor blockers on insulin secretion in the rat. Acta Endocrinologica105 (1): 78–82.

Bilbis L.S., Muhammad, S.A., Saidu, Y., & Adamu, Y. (2012). Effect of vitamins A, C, and supplementation in the treatment of metabolic syndrome in albino rats .Biochemistry Research International, 1-7.

Boschmann, M., Krupp, G., Luft, F.C., Klaus, S., and Jordan, J. (2002). In Vivo Response to α 1 -Adrenoreceptor Stimulation in Human White Adipose Tissue. Obesity Research 10 (6): 555–558.

Cheng, J.T., Liu, I.M., Yen, S.T., & Chen, P.C. (2000). Role of alpha1A-adrenoceptor in the regulation of glucose uptake into white adipocyte of rats in vitro. Autonomic Neuroscience : Basic & Clinical 84 (3): 140–6.

Chika, A., Onyebuece, D.C., & Bello S.O. (2018). Phytochemical analysis and evaluation of antidiabetic effects in alloxan-induced diabetic rats treated with aqueous leaf extract of *Acanthospermum hispidium.* African Research Journal of Biomedical research 21:81-85.

Dikko, M., Bello, S.O., Chika, A., Mungadi I.A, Sarkingobir Y, & Aliyu, S. (2020a). Determination of Oral Glucose Tolerance (OGT) of Benign Prostatic Hyperplasia Patients Treated with Tamsulosin in Sokoto State, Nigeria. Nigerian Journal of Pharmaceutical and Applied Science Research, 9(2): 33-39.

Dikko, M. (2019). Exploration of gross effect of tamsulosin on glucose and insulin kinetics in ratsand humans. A PhD thesis submitted to the Postgraduate School Usmanu Danfodiyo University Sokoto, Nigeria.

Dikko, M., Bello, S.O., Chika, A, Mungadi, I.A., Sarkingobir, Y., & Umar, AI (2020b). Effect of Tamsulosin use on plasma insulin status in Benign Prostatic Hyperplasia patients in Sokoto, Nigeria. Journal of Applied Science and Environmental Management, 24 (4) 543- 548.

Flechtner-Mors, M., Jenkinson, C.P., Alt, A., Biesalski, H.K., Adler, G., & Ditschuneit, H.H. (2004). Sympathetic Regulation of Glucose Uptake by the α 1 -Adrenoceptor in Human Obesity. Obesity Research 12 (4): 612–620.

Jacob, S., Rett, K., Wicklmayr, M., Agrawal, B., Augustin, H.J., & Dietze, G.J. (1996). Differential effect of chronic treatment with two beta-blocking agents on insulin sensitivity: The carvedilol-metoprolol study. Journal of Hypertension14(4): 489–494.

Jerry, J. (2018). Glucose tolerance testing. Www.medicine.medscape.con. Retrieved 7/5/2020

Onakpoya, I.J., Heneghan, C.J., & Aronson, J.K. (2016). Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: A systematic review of the world literature. BioMed Central Medicine14 (1): 10.

Patrick, J.P. (2012). Oral glucose tolerance testing. Australian Family Physician, 41(6):391-193.

Satin, L.S. (2000). Localized calcium influx in pancreatic β-cells: Its significance for Ca2+-dependent insulin secretion from islets of Langerhans. Endocrine13 (3): 251–262.

Shivaprasad, G.M., Bharatha, A., Naikwadi, A.A., & Wali R.S. (2015). Effect of tamsulosin a selective α1 -antagonist on glucose homeostasis in rats. International Journal of Pharmacy and Pharmaceutical Sciences 7 (3): 232–234.

Suresha, R.N., Ashwini, V., Pragathi, B., Kalabharathi, H.L., Satish, A.M., & Pushpa, V.H. (2013). The effect of carvedilol on blood glucose levels in normal albino rats. Journal of Clinical and Diagnostic Research 7 (9): 1900–1903.

Uduala, T., Umar R.A., Isah R.A., Bello, M., Aiyelabegan, Isa, L.O., & Oduala G.B. (2018). Use of Gliricidia sepium aqueous leaf extracts as an antisickling agent: Oxidative stress biomarkers in wistar rats exposed to the extract. International Journal of Medical and Health Research4(8):79-83.

Umar, R. A., Hassan, S.W., Ladan, M.J., Matazu, I.K., Shehu, B., Shehu, R.A., Muhammed, L.G., & Molabo, F.I. (2010). Adverse effect associated with administration of antiretroviral drugs (Nevirapine, Lamivudine and Stavudine) to albino rats: Implication for management of patients with HIV/AIDS. Asian Journal of Biochemistry 5(3): 181-187.

Umar, M.T., Bello, S.O., Chika, A., & Oche, O.M. (2016). Attitude of nurses and pharmacists on adverse drug reactions reporting in selected hospitals in Sokoto. Journal of Research in Pharmacy Practice 5:219-2121.

United States National Library of Medicine (2020). Glucose tolerance test: Non pregnant .www.medlineplus.gov. Retrieved 7/5/2020

Woodcock, J. (2016). “Precision” drug development? Clinical Pharmacology & Therapeutics99 (2): 152–154.

World Health Organisation (2005). WHO Draft Guidelines for Adverse Event Reporting and Learning Systems. Geneva, Switzerland: Author. Report No. 80: Retrieved March 16, 2019.

World Health Organisation (2006). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. WHO, 20 Avenue Appia, 121 Geneva 27, Switzerland.

Niger. J. Physiol. Sci. 35 (December 2020): 191 - 195
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Effect of Furosemide on Dexmedetomidine-Ketamine Anaesthesia in Cats

***Oguntoye C.O. and Akande C.Y.**

Department of Veterinary Surgery and Radiology, University of Ibadan, Ibadan

Summary: The effects of intramuscular (IM) administrations of 10mcg/kg dexmedetomidine, followed 10 minutes later by either IM injection of 10mg/kg ketamine alone (DK) or with 2.5 mg/kg furosemide (DKF) were assessed in five healthy cats (3 males and 2 females) using selected anaesthetic indices (Time to onset of anaesthesia (OA), Duration of Analgesia (DA), Duration of Recumbency (DR), and Time to Standing (TS), as well as, changes in heart rate (HR), respiratory rate (RR) and rectal temperature (RT) , following loss of righting reflex, and at 10 min intervals for 60-minute. The OA for DKF group (2.2 ±0.45 min) was not significantly (P>0.05) different from that for DK group (2.4± 1.14 min). The DA (42.6 ±13.01min) and DR (71.6 ±17.94min) for DKF group were longer than respective values of DA (31.8± 14.3) and DR (51.2 ± 16.2min) for the DK group. The TS for DKF (3.6 ± 2.8 min) was shorter than TS (8.0±3.8) for DK. However, these differences were not statistically significant (P > 0.05). HR, RR and RT were from 84.8 ± 8.7 to 113.2±30.7 beats/min, 17.4± 6.2 to 48.8 ±12.1 breaths/min and from 36.0±0.5 to 37.6 ± 0.6°C (DKF); 96.0±19.4 beats/min, 24.8±19.1 to 71.2 ± 34.3 breaths/min and from 36.0±0.5 to 37.6 ± 0.6°C (DKF); 96.0±19.4 to 112.8± 44.3 beats/min, 24.8±19.1 to 71.2 ± 34.3breaths/min and from 35.1±1.2 to 37.6 ±0.8 °C (DK). There were no significant differences (P > 0.05) in the vital parameters between the DKF and DK treatments. The values for HR and RR for DKF were generally lower than those for DK group. It was concluded that concurrent administration of furosemide with dexmedetomidine- anaesthesia in cats prolonged the duration of analgesia and recumbency but had no effect on onset of anaesthesia. A cat on this anaesthetic combination concurrently placed on furosemide medication will therefore need to be carefully monitored until full recovery.

Keywords*: Anaesthesia, Cat, Combination, Dexmedetomidine, Ketamine, Furosemide*

**Address for correspondence: wumcel06@gmail.com; Tel: +234-8055063671*

Manuscript received: September 2019. Revised version accepted: June, 2020

INTRODUCTION

Ketamine hydrochloride is a phencyclidine derivative which is commonly used in both human and veterinary medicine (Mercer *et al* ., 2009; Kurdi *et al* ., 2014; Berry, 2015). Unlike other currently used anaesthetic agents, ketamine stimulates the cardiovascular system thereby producing increased heart, arterial blood pressure and cardiac output (Kistner, 2018). Ketamine also has minimal effects on central respiratory drive and produces airway relaxation by acting on various receptors, inflammatory cascades and bronchial smooth muscles (Berry, 2015). Ketamine is licensed for use as a sole anaesthetic agent for cats and non-human primates in some countries (Selmi *et al* .,2004; Kistner, 2018). However, because it is associated with increased muscle tone, involuntary movement and a high incidence of excitation during recovery, it is usually combined with a tranquilizer or sedative. (Clarke *et al* ., 2014; Berry. 2015; Kistner, 2018).

Ketamine combination with α2-adrenoceptor agonists for anesthesia in cats is well established (Selmi *et al* ., 2003; Thomas and Lerche, 2011; Clarke *et al* ., 2014). Dexmedetomidine, the active enantiomer of medetomidine, is the latest α2 agonist sedative. It is reportedly twice as potent as medetomidine, more selective for α2- receptors than xylazine and thus has fewer side effects (Ansah *et al* ., 1988; Kuusela *et al* ., 2000; Thomas and Lerche, 2011; Carvahalo *et al* ., 2019). Dexmedetomidine compensates for the poor muscle relaxant and analgesic effects of ketamine, while the cardiac stimulating properties of ketamine partially compensate the dexmedetomidine-induced bradycardia (Haskins *et al* ., 1986; Verstegen *et al* ., 1988). Both dexmedetomidine and ketamine can be administered subcutaneously, intravenously and intramuscularly, thus making the combination useful in feline practice for procedural sedation and anaesthesia especially for difficult to handle or feral cats using the intramuscular route (Clarke *et al* ., 2014; Kistner, 2018).

A cat under dexmedetomidine - ketamine anaesthesia may need to be administered a diuretic for various reasons. Cats are small in size and prone to fluid overload which may need to be treated with a diuretic. Also, a cat on furosemide for congestive heart failure may require ketamine anaesthesia.

In cats, ketamine is predominantly excreted as an unchanged metabolite and directly excreted by the kidney (Berry, 2015). In most other species, metabolism of ketamine occurs in the liver. It is demethylated by hepatic microsomal enzymes, reducing the active metabolite norketamine. Norketamine is hydroxylated and then conjugated to form water-soluble and inactive glucuronide metabolites that are excreted by the kidney (Stoelting, 1999). In cat, however, ketamine is biotransformed by hepatic microsomal enzymes to norketamine, which is

excreted unchanged in the urine (Hanna *et al* ., 1988; Berry, 2015; Kistner, 2018). Possible drug interaction may therefore be expected when a diuretic and dexmedetomidine - ketamine combination is administered. A few studies have investigated drug-drug interactions between diazepam-ketamine and xylazine- ketamine anaesthesia and furosemide in both cats and rabbits with varying results (Adetunji *et al* ., 2010; 2013). There is, however, a paucity of information in literature, on furosemide concurrent administration during dexmedetomidine-ketamine anaesthesia in cats. The aim of this study, therefore, was to evaluate the effects of furosemide on dexmedetomidine-ketamine anaesthesia in cats.

MATERIALS AND METHODS

Animals: Five adult domesticated, home-bred, short-haired intact local cats (2 females and 3 males) acquired from a local market in Ibadan, Nigeria, mean body weight ± SD of 1.59 ± 0.0kg were studied.

Animal Housing: The cats were housed at animal house (Cat section) of the Veterinary Surgery and Radiology Department, University of Ibadan, in clean, well aerated compartments and with beddings and sand boxes.

Animal Stabilization: The cats were acclimatized for a period of 4 weeks in order to get them adjusted to the new environment, feeding regime and handling. The cats were given oral anthelminthic, pyrantel pamoate (Pyranthrin®, Neimeth pharmaceuticals, Oregun, Nigeria) supplied as 250mg/ml suspension at a dosage of 5mg/kg body weight. They were fed with home-made food comprising fish, commercial cat food (Binggo®, Grand Cereals, Nigeria) egg, rice, solid pap and palm-oil twice daily and water was provided ad-libitum. Just before the commencement of the experiments they were judged to be in good health based on result of comprehensive clinical examination.

Trial Drugs: The experimental drugs were-

1) Furosemide (Lasix®, Philomide), supplied as a 10mg/ml solution for intramuscular or intravenous injection in a 2 ml ampoule.
2) Dexmedetomidine (Dexdomitor®; Orion Corporation, Orionintie 1 FI-02200 Espoo, Finland) supplied as a 0.1mg/ml solution for intramuscular or intravenous injection in a 15-ml multi dose vial.
3) Ketamine hydrochloride (Ketalar®; Kwality Pharmaceuticals Pvt. Ltd, Amritsar-India) was supplied as a 50mg /ml aqueous solution for intramuscular or intravenous injection in a 10-ml multi-dose vial.

The drugs were administered with 1ml or 2ml syringe.

Study design: The cats (3 males and 2 female) were employed in the two set of trials at one-week interval. In the first set of trials (DK) dexmedetomidine was administered as a pre-medicant, followed after 10 minutes, by ketamine. In the second trial (DKF), the cats were premedicated with dexmedetomidine followed after 10 minutes by simultaneous administration of ketamine and furosemide. The cats' physiological parameters were taken every 10 minutes for 60 minutes. Selected anaesthetic indices were recorded.

Trial drugs administration and monitoring: Food was withheld from the cats 12 hours prior to drug administration, but they were allowed free access to water. The cats body weights were then determined using a weighing scale (Camry scales, China). The DK group had an intramuscular injection of 10 mcg/kg followed ten minutes later with intramuscular administration of ketamine at a dosage of 10 mg / kg. After 7days (allowance for wash-off), the second trial (DKF) commenced with intramuscular injection of dexmedetomidine (10 mcg/kg), followed 10 minutes later with intramuscular administration of ketamine and furosemide at the dose rate of 10mg/kg and 2.5mg/ kg respectively.

Following loss of righting reflex, each cat was placed on lateral recumbency and allowed to breathe in-room air for the duration of the study. A pedal withdrawal reflex following paw-pinch with artery forceps applied at interdigital skin and closed at the third ratchet was used to evaluate loss of pain sensation. The evaluation was done immediately after ketamine administration and repeated at two-minute intervals until the pedal withdrawal reflex reappeared.

Measurements of physiological variables: Following the loss of righting reflex by the cats the heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were determined and thereafter at 10 minutes' interval over a 60-minute period. The heart rate (in beats per minute) was determined with the aid of a precordial stethoscope. Respiratory rate (in breaths per minute) was determined by counting the cat's chest movement while rectal temperature (in °C) was determined using a mercury-in- glass clinical thermometer.

Calculations: The following anaesthetic indices were calculated:

a) Onset of anaesthesia: time interval (in minutes) between ketamine administration to loss of righting reflex.
b) Duration of analgesia: time interval (in minutes) between loss of pedal reflex and return of pedal reflex.
c) Duration of recumbency: time interval (in minutes) between loss of righting reflex and assumption of sternal posture.
d) Time to standing: time interval (in minutes) between assumption of sternal posture and time to stand.

Statistical analysis:

All data were expressed as means ± standard deviation (SD). The means of the anaesthetic indices between the DK and DKF groups were compared using student's t-test for paired data. The respective means of the HR, RR and RT were compared using analysis of variance for repeated measures followed as appropriate by Tukey-Kramer multiple comparisons test (Dawson and Trapp, 2004). A p-value of less than 0.05 was accepted for statistical significance in all comparisons.

RESULTS

Observation: All the cats reacted to pain on intramuscular injection of the drugs. Two cats, one each in the Dexmedetomidine/Ketamine (DK) and

Dexmedetomidine/Ketamine/Furosemide (DKF) groups defecated some minutes after ketamine administration. Salivation was observed in two cats in the (DK) group. In the (DKF) group, a cat salivated while two others vomited.

Anesthetic indices: The selected anesthetic indices that were calculated are shown in Table 1. Time to onset of anaesthesia in the cats with DK (2.4 ± 1.14 min) was similar to that with DKF (2.2±0.45 min) (p = 0.729). The duration of analgesia with (DK) 31.8 ± 14.31 min was not significantly different (p = 0. 247) from that with DKF (42.6±13.01 min). The duration of recumbency with DKF, though longer, (71.60±17.94 min) but not significantly different from that with DK (51.20±16.24 min) with a p-value of 0.096. Time to standing was shorter with DKF (3.6±2.80 min), but was not significantly different when compared with DK (8.0±3.81 min) (p = 0.073).

Table1:
Showing selected anesthetic indices of the intramuscular administration of dexmedetomidine/ketamine and dexmedetomidine/ketamine /furosemide in cats studied.

Treatment groups	Anaesthetic Indices (min)	
	D-K[a]	D-K-F[b]
Onset of Anaesthesia	2.4 ±1.14	2.2 ± 0.45
Duration of Analgesia	31.8 ±14.31	42.6±13.01
Duration of Recumbency	51.2 ± 16.24	71.6 ±17.94
Time to Standing	8.0 ± 3.81	3.6 ±2.80

Data are expressed as means ± standard deviation
*a) **DK**: Dexmedetomidine/Ketamine group (10mcg/kg of dexmedetomidine-10mg/kg of ketamine)*
*b) **DKF:** Dexmedetomidine/Ketamine/Furosemide group (10mcg/kg of dexmedetomidine-10mg / kg of ketamine-2.5mg/kg of furosemide*)

Physiological parameters
The mean heart rate, respiratory rate and temperature of the cats following the intramuscular administration of DK and DKF are shown in Table 2.
Heart rates: Mean heart rates with DK ranged from 96.0±19.39 to 112.8±44.3 beats/min while that of DKF ranged between 84.8±8.67 and 113.2 ±30.71 beats/min. There was no significant difference (p between the two groups.
*Respiratory rate***:** Mean respiratory rates of the group DK ranged from 24.8±19.1 to 71.2 ±34.3 breaths/min while that of DKF ranged from 17.4 ±6.15 to 48.8 ± 12.13 breaths/min. Although there was no significant difference (p>0.05) in the respiratory rates between the two treatments, the values were generally lower in the DKF group.
*Rectal temperature***:** Mean rectal temperatures with DK ranged between 35.1±1.24 and 37.62 ±0.75°C and 36.0 ± 0.54 to 37.64± 0.63°C with DKF. There were no significant differences in mean RT between the two groups(p>0.05) though values fell from the 40th minute of anaesthesia.

DISCUSSION

The observed vomiting by two cats following DKF administration was consistent with emetic effect of alpha 2 agonists in this species (Granholm, 2006, 2007; Thomas and Lerche; 2011; Robertson *et al* ., 2018; Carvahalo *et al* ., 2019). Many cats vomit following administration of dexmedetomidine especially with high doses (Robertson *et al* ., 2018). The dose rates of both dexmedetomidine and ketamine used in this study were those recommended in literature (Neto, 2009). However, dexmedetomidine is associated with fewer side effects than the older α2 agonist, xylazine (Thomas and Lerche,2011). Salivation was also reported in cats given dexmedetomidine- ketamine combination (Carvahalo *et al* ., 2019). Salivation in some of the cats in our study may therefore not be attributable to ketamine alone (Clarke *et al* ., 2014; Thomas and Lerche, 2011) but to both ketamine and dexmedetomidine effects. Anticholinergic (atropine) is useful in preventing or treating older α2 agonist associated salivation in dogs, but it's use in cats is associated with the production of thick mucous secretions within the airways and this viscous secretion may predispose the patient to airway blockage (Thomas and Lerche, 2011). Furthermore, anticholinergics may elicit prolonged tachycardia with α2-agonist – ketamine combinations (Alvaides *et al* ., 2008; Thomas and Lerche, 2011). Routine anticholinergic administration to prevent dexmedetomidine induced bradyarrhythmias is also contraindicated because it can cause significant hypertension (Montero *et al* ., 2009) and may be associated with premature ventricular depolarizations (Alvaides *et al* ., 2008). Both dexmedetomidine and ketamine cause pain on intramuscular injection as observed in these cats (Thomas and Lerche, 2011). The result of this study showed similar anaesthetic induction times in the cats to DK (2.4± 1.14 min) and DKF (2.2 ± 0.45min). Similar studies in rabbits with xylazine- ketamine, and in cats with diazepam/xylazine-ketamine (Adetunji *et al* ., 2010, 2013) also reported that administration of furosemide with the sedative- ketamine combinations did not have any influence on anaesthetic induction times.

Table 2:
Showing the heart rate, respiratory rate and rectal temperature responses of the cats to intramuscular administration of dexmedetomidine-ketamine alone (DK) and dexmedetomidine-ketamine-furosemide (DKF).

Time interval	HR(beats/min)		RR (breaths/min)		RT(^{0}C)	
(min)	DK	DKF	DK	DKF	DK	DKF
0[a]	100.4±38.5	112.2±11.3	40.9±23.3	48.8±12.1	37.0±0.7	37.5±0.9
10	111.6±28.8	109.0±5.7	28.8±27.3	17.4±6.2	37.6±0.7	37.6±0.6
20	96.0±19.4	89.0±11.5	24.8±19.1	19.8±6.7	37.5±0.9	37.0±0.6
30	97.6±2.9	84.8±8.7	36.4±27.6	29.2±10.0	37.9±1.4	37.0±0.6
40	112.8±44.3	87.2±14.8	36.8±27.6	36.8±17.3	36.1±1.4	36.0±0.5
50	105.5±28.1	94.0±26.1	39.6±25.4	34.0±13.3	36.6±1.2	36.8±0.6
60	110.4±32.9	87.0±14.5	71.2±34.3	48.4±15.0	35.1±1.2	36.4±0.6

Data were expressed as means ± SD

Nonetheless, the concurrent administration of furosemide to the dexmedetomidine- ketamine anaesthetic combination produced longer duration of analgesia (DKF- 42.6 ± 13.01 min; DK- 31.8±14.31min) and recumbency (DKF- 71.6 ± 17.94 min; DK- 51.2±16.24 min) but a shorter standing time (DKF- 3.6 ± 2.8 min; DK- 8.0±3.81min) than dexmedetomidine-ketamine alone (Table 1). The greater duration of recumbency associated with the DKF group than the DK values in this study is consistent with the findings of Hanna and others (1988) who reported that, on the basis of measured pharmacokinetic parameters, the concurrent use of diuretics such as furosemide prolonged the renal excretion of ketamine in cats. In a similar study in rabbits, inclusion of furosemide with xylazine-ketamine anaesthetic combination also produced a longer duration of recumbency (Adetunji *et al* ., 2013). In addition, the duration of recumbency of 51.2±16.24 min obtained for the DK is similar to 51.6 ±13.5 min recorded following administration of dexmedetomidine-ketamine combination in cats by Selmi and others (2003).

The heart rates obtained from the cats with both DK and DKF treatments did not show any significant differences ($p > 0.05$) although the DKF (84.8±8.67 and 113.2 ±30.71 beats/min) values were generally lower than the values for DK (96.0±19.39 to 112.8±44.3 beats/min) (Table 2). Acceptable heart rate of cats under general anaesthesia is about 100 beats/minute (McKelvey and Hollingshead, 2000; Kennedy and Johnson, 2015) although values of 60-120 beats/min are usually seen (McKelvey and Hollingshead, 2000). Whereas α2-agonists are known to produce bradycardia, ketamine stimulates the cardiovascular system (Kistner, 2018). The possibility of ketamine partially counteracting the α2 adrenoceptor agonist-induced bradycardia and hypotension has been suggested (Haskins, 1988; Verstegen, 1991). The lowest heart rates recorded following both DK and DKF treatments were close to 88 beats/min obtained in a similar study (Selmi *et al* ., 2003). The administered a2-agonists alone produced a minimal respiratory effect in healthy dogs and cats, characterized by a decrease or no change in respiratory rate and minimal blood gas tension (Berry, 2015). However, significant hypoventilation resulting in hypoxia can occur when a2-agonists are administered with other drugs like opioids, ketamine or propofol (McDonnel and Kerr, 2007). The respiratory rates with both treatments in this study (Table 2) were consistent with possible rates of up to 50 breaths/minute especially with moderate anaesthetic depth (Mckelvey and Hollingshead, 2000).

The mean rectal temperatures obtained following both DK and DKF treatments were similar (Table 2) and fell within the normal temperature range of 36.7- 38.9°C in cats (Levy *et al* ., 2015) until the 60th minute in DK treatment when the cats became hypothermic (35.1±1.24°C; Table 2). It is therefore suggested that warming devices to treat hypothermia should be made available with the use of this combination.

It was concluded that concurrent administration of furosemide with dexmedetomidine- anaesthesia in cats prolonged the duration of analgesia and recumbency but had no effect on onset of anaesthesia. A cat on this anaesthetic combination concurrently placed on furosemide medication will therefore need to be carefully monitored until full recovery.

REFERENCES

Adetunji, A., Lawal, F. M. and Oguntoye, C. O. (2013). The effect of concurrent administration of furosemide on xylazine/ketamine anaesthesia in rabbits. NVJ. 34 (4): 863-869.

Adetunji, A., Oguntoye, C. O., Lawal, F.M. and Thani, V. O. (2010). The effect of concurrent administration of furosemide on diazepam/ketamine anaesthesia in cats. In: Proceedings of the 47th Annual Congress of the Nigerian Veterinary Medical Association. 70 -73.

Alvaides R. K., Teixeira Neto, F. J., Aguiar, A. J. A. and Steagall, P. V. (2008). Effects of acepromazine or atropine given before dexmedetomidine in dogs. Vet. Rec. 162 (10) :852-856. and Physiology in Anesthetic Practice, 3rd edn. Williams & Wilkins, Philadelphia: Lippincott, pp 140–157.

Ansah, O. B., Raekallio, M. and Vainio, O. (1998). Comparison of three doses of dexmedetomidine with medetomidine in cats following intramuscular administration. J. Vet. Pharmacol. Ther. 21: 380–387.

Berry, S. H. (2015). Injectable anaesthetics. In: Tranquilli, W. J., Thurmon, J. C. and Grimm, K. A. (eds.) Lumb and Jones' Veterinary Anesthesia and Analgesia. 4th edn. Blackwell, Ames, Iowa, U.S.A., pp 297-331.

Carvalho, E. R., Champion, T., Vilani, R. G. O. C., Freitas, G. C., Ambrosini, F., Silva, G. A., Gonçalves, K. S. and Fischborn, J. C. J. (2019). Sedative and electrocardiographic effects of low dose dexmedetomidine in healthy cats. Pesq. Vet. Bras. 39 (2):142-147.

Clarke, K. W., Trim, C. M. and Hall, L. W. (2014). General Pharmacology of the injectable agents. In: Clarke, K. W., Trim, C. M. and Hall, L. W. Veterinary Anaesthesia. Saunders Elsevier Ltd., London, pp135-148.

Dawson, B. and Trapps, R. G. (2004). Basic and clinical biostatistics, 4th edition, McGraw Hill, New York, pp 162-189.

Granholm, M., McKusick, B. C., Westerholm, F. C. and Aspegrén, J. C. (2006). Evaluation of the clinical efficacy and safety of dexmedetomidine or medetomidine in cats and their reversal with atipamezole. Vet. Anaesth. Analg. 33(4): 214-223.

Granholm, M., McKusick, B. C., Westerholm, F. C. and Aspergen, J. C. (2007) Evaluation of the clinical efficacy and safety of intramuscular and intravenous doses of dexmedetomidine and medetomidine in dogs and their reversal with atipamezole. Vet. Rec. 160 (26):891-897.

Hanna, R. M., Borchard, R. E. and Schmidt, S. L. (1988). Pharmacokinetics of ketamine HCl and metabolite I in the cat: a comparison of I.V., I.M., and rectal administration. J. Vet. Pharmacol. Ther. 11(1):84-93.

Haskins, S. C., Patz, J. D., Farver, T. B. (1986). Xylazine and xylazine-ketamine in dogs. Am. J. Vet. Res. 47:636–641.

Kennedy, M. J. and Johnson, R. A. (2015). Dexmedetomidine and Atipamezole Clinician's Brief. 65-67

Kistner, S. B. R. (2018). Injectable anaesthetics. In: Duke-Novakovski, T., Marieke de Vries and Seymour, C. (eds)

BSAVA Manual of Canine and Feline Anaesthesia and Analgesia 3rd Edition British Small Animal Veterinary Association, Quedgeley, Gloucester, pp 190 - 213

Kurdi, M. S., Theerth, K. A. and Deva, R. S. (2014). Ketamine: Current applications in anesthesia, pain, and critical care. Anesth Essays Res. 8(3): 283–290.

Kuusela, E., Raekallio, M. and Vainio, O. (2000). Comparison of three doses of dexmedetomidine and its enantiomers in dogs. J. Vet. Pharmacol. Ther. 23:15–20.

Levy, J. K., Nutt, K. R. and Tucker, S. J. (2015). Reference interval for rectal temperature in healthy confined adult cats. J. Feline Med. Surg. 17(11):950-952.

McDonell, W. N. and Kerr, C. L. (2007). Respiratory system. In: Tranquilli, W. J., Thurmon, J. C. and Grimm, K.A. (eds.) Lumb and Jones' Veterinary Anesthesia and Analgesia. 4th ed. Blackwell, Ames, Iowa, pp 117-152.

McKelvey, D. and Hollingshead, K. W. (2000). General anaesthesia. In: McKelvey, D. and Hollingshead, K.W. (eds.) Veterinary Anesthesia and Analgesia. Mosby, St. Louis. Missouri, pp 51-110.

Mercer, S. J. (2009). 'The Drug of War' – a historical review of the use of Ketamine in military conflicts. J. Roy. Naval Med. Serve. 95: 145–150.

Monteiro, E. R., Campagnol, D., Parrilha, L. R. and Furlan, L. Z. (2009) Evaluation of cardiorespiratory effects of combinations of dexmedetomidine and atropine in cats. J Feline Med. Surg. 11: 783–792.

Neto, F. J. T. (2009). Dexmedetomidine: a new alpha-2 agonist for small animal practice Proceedings of the 34th World Small Animal Veterinary Congress WSAVA 2009, São Paulo, Brazil.

Robertson, S. A., Gogolski, S. M., Pascoe, P., Shafford, H. L., Sager, J. and Griffenhagen, G. M. (2018) J. Feline Med. and Surg. 20: 602–634.

Selmi, A. L., Mendes, G. M., Lins, B.T., Figueiredo, J. P. and Barbudo-Selmi, G. R. (2003). Evaluation of the sedative and cardiorespiratory effects of dexmedetomidine, dexmedetomidine-butorphanol and dexmedetomidine-ketamine in cats. J. Am. Vet. Med. Assoc. 222:37–41.

Selmi, A. L., Mendes, G. M., Figueiredo, J. P., Barbudo-Selmi., G. R. and Lins, B. T. (2004). Comparison of medetomidine-ketamine and dexmedetomidine-ketamine anesthesia in golden-headed lion tamarins. Can. Vet. J. 45: 481-485.

Stoelting R. K. (1999). Non barbiturate induction drugs. In: Stoelting, R. K. (ed.) Pharmacology, Thomas, J. A. and Lerche, P. (2011): Anesthetic agents and adjuncts. In: Anesthesia and Analgesia for Veterinary Technicians 4th Edn Mosby Elsevier, Missouri, USA, pp 50 -95.

Verstegen J., Fargetton X., Ectors F. (1989) Medetomidine-ketamine anaesthesia in cats. Acta Vet Scand; 85:117–123.

Verstegen J., Fargetton X., Donnay I., Ectors F. (1991): An evaluation of medetomidine/ketamine and other drug combinations for anaesthesia in cats. Vet. Rec. 128 (2) :32–35.

Niger. J. Physiol. Sci. 35 (December 2020): 195 - 201
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Attenuating Effect of *Telfairia occidentalis* on Oxidative Stress in Indomethacin-induced Gastric Ulcer in Male Rats

Adisa W.A.[1], Otamere H.O.[1], Adesina A.A.[2], Bankole J.K.[4]

Departments of [1]Physiology, [2]Psychology and [3]Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State,Nigeria.

Summary: Peptic ulcer is a major health challenge with high morbidity and mortality all over the world. This study investigated the involvement of oxidative stress in the healing and protective potentials of aqueous leave extract of *Telfairia occidentalis* (*TO*) on indomethacin induced gastric ulcers in adult Sprague Dawley male rats. The rats were divided into 6 groups (A-F) of 5 rats each, with A as normal control, B received single oral administration of 40mg/kg indomethacin without treatment for 4 hours; C received 40mg/kg indomethacin without treatment for 4 hours and scarified after 72 hours; D received 100mg/kg aqueous leave extract of TO for 7 days without ulcer induction; E (pre-treated test group) received 40mg/kg indomethacin after being pre-treated with 100mg/kg aqueous leave extract of TO daily for 7 days. Group F (Post treated test) received 40mg/kg of indomethacin and treated four hours later with 100mg/kg aqueous leave extract of TO daily for 7 days. The results revealed changes in gastric macroscopic architecture of the mucosa, and changes in ulcer indices and oxidative stress markers levels in group B-F. These changes comparatively suggested that the leave-extract of *Telfairia occidentalis* has gastro-protective with minimal healing potentials mediated through reduced oxidative stress.

Keywords*: Telfairia occidentalis, gastric ulceration, MDA, indomethacin*

**Address for correspondence: williamsadewumi@yahoo.com; Tel: +234-8056049158*

Received – July, 2020; Accepted- October 2020

INTRODUCTION

Gastric ulcer is one of the major causes of morbidity and mortality (Chaturvedi *et al*., 2007; Miami *et al*., 2016) and has remained a worrisome health challenge (Lanas and Chan, 2017), with known capacity to induce gastrointestinal bleeding when not adequately treated (Tortora and Grabowski, 2003; Leontiadis *et al*., 2013; Lanas *et al*. 2014). It has been reported that the etiology of gastroduodenal ulcers are caused by imbalance between offensive factors such as gastric HCl, pepsin secretion; and defensive factors such as parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (Repetto and Llesuy, 2002). According to Malyshenko *et al*. (2005), factors like inadequate dietary habits, cigarette smoking, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may also be responsible for the development of peptic ulcers. Similarly, Reactive Oxygen Species (ROS) has been implicated as one of the major causes of mucosal lesions and gastrointestinal damage (Bagchi *et al*., 1999; Pohle *et al*., 2001; Whittle, 2003).

Noteworthy is the fact that several pharmaceutical products have been employed in the treatment of gastro-duodenal ulcer and peptic diseases, resulting in decreasing mortality and morbidity rates; but considering their associated adverse effects, such products cannot be described as completely effective (Rates, 2001; Wedemeyer and Blume, 2014). One widely used drug that has been associated with rare idiosyncratic hepatotoxicity is the histamine H2 receptor antagonist-ranitidine (Bourdet *et al*., 2005). It is available over the counter for oral administration or by prescription for parenteral administration for treatment of gastric ulcers, hypersecretory diseases, and gastroesophageal reflux disease. Idiosyncratic ranitidine hepatotoxicity occurs in few people taking the drug (Fisher and Le Couteur, 2001). Most liver reactions are mild and reversible; but extensive liver damage has occurred in individuals undergoing ranitidine therapy (Ribeiro *et al*., 2000). Thus, confirming the adverse effects earlier alluded to. Nevertheless, with the onset of scientific research in medicinal herbs, it is becoming clearer that medicinal herbs have potentials of great importance in this regard (Kurekci *et al*., 2012).

Indeed, there has been growing interest in alternative therapies and the use of natural products, especially those derived from plants (Schmeda-Hirschmann and Yesilada, 2005). Studies on the treatment of gastrointestinal disorder have focused on the potential role of natural medicine due to their availability, better protection, lower cost, and lower toxicity (Bansal and Goel, 2012). Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcers (Jung *et al*., 2019; Morufu *et al*., 2014). One of such plants that has been extensively studied and reported to have great potential is *Telfairia occidentalis* –the plant on focus in this study.

Telfairia occidentalis commonly called fluted pumpkin occurs in the forest zone of West and Central Africa, most frequently in Benin, Nigeria and Cameroon. It is a popular vegetable all over Nigeria. It has been suggested that it originated from South-East Nigeria, and distributed by the

Igbos who have cultivated it crop since time immemorial (Kayode and Kayode, 2011). It is a vigorous perennial vine, growing to 10m or more in length. The stems have branching tendrils and the leaves are divided into 3– 5 leaflets. The fruits are pale green, 3 – 10 kg in weight, strongly ribbed at maturity and up to 25cm in diameter. The seeds are 3– 5cm in diameter (FAO 1989). The leaf is consumed in different parts of the country because of the numerous nutritional and medicinal attributes ascribed to it. *Telfairia occidentalis* seeds serve as a high quality and low cost plant protein source for animal feed formulations (Kuku *et al*., 2014). It has different traditional names: "Ugu" in Igbo, "Iroko" or Aporoko in Yoruba, Ubong in Efik, Umee in Urhobo and Umeke in Edo (Akoroda, 1990). Young succulent shoots and leaves are used as vegetables in the eastern part of Nigeria. Apart from its nutritional value, the plant also has agricultural and industrial importance (Oboh, 2005).

Medicinally, the herbal preparation of the plant has been employed in the treatment of sudden attack of convulsion, gastrointestinal disorders, malaria and anaemia (Akindele *et al*., 2013; Gbile, 1986). Quite a number of researchers in the field of medical sciences have observed free radical scavenging ability and antioxidant property in *Telfairia occidentalis*. The darkish green leafy vegetable of *Telfairia occidentalis*, and its extracts (such as aqueous and ethanol extracts), have been found to suppress or prevent the production of free radical and/or scavenge already produced free radicals, lower lipid peroxidation status and elevate antioxidant enzymes (such as superoxide dismutase and Catalase) both in vitro and in vivo (Kayode *et al*., 2010; 2009; Oboh *et al*., 2006; Oboh and Akindahunsi, 2004). Studies have also shown that the leaves of *Telfairia occidentalis* are rich in antioxidants such as ascorbic acid and phenols (Oboh, 2005; Oboh and Akindahunsi, 2004). Toyin *et al*. (2013) and Adisa *et al*. (2018) had also reported the blood glucose-lowering effects of *Telfairia occidentalis* and its potentials in the management blood glucose levels.

Before the discovery of H pylori it was known that patients with duodenal ulcers secrete about twice as much acid as controls because they have twice as many parietal cells (Calam and Baron, 2001). Patients with gastric ulcer and those with functional dyspepsia have normal acid output and parietal cell count (Calam and Baron, 2001). Thus, there was good evidence that acid played a major role in ulcer formation (Calam and Baron, 2001).

Although several studies have been conducted on the medicinal potentials of *Telfairia occidentalis*, its pharmacological effects on gastrointestinal system is yet to be adequately explored. Adisa *et al*. (2019), reported ameliorative effect of *Telfairia occidentalis* on induced gastric ulcer; worrisome however, is that its ingestion may increase gastric acidity and hence this research to investigate further; the effect of *Telfairia occidentalis* leave extract on indomethacin induced gastric ulcer in rats.

MATERIALS AND METHODS

Materials (Plant, drugs and feed): *Telfairia occidentalis* leaves commonly called pumpkin leaves were obtained from a local market in Ekpoma, Edo State and taken to the herbarium unit of the Department of Botany of Ambrose Alli University, Ekpoma for identification.

Indomethacin (B.P. 25mg; Manufacture by Fabrique Par, Yangzhau Pharmaceutical Co. LTD. Yiling-Jiangdu, China) was purchased from a pharmacy in Ekpoma, Edo State, Nigeria. the animal feed (grower's mash; Grower palletised produced by Grand Cereals Ltd, a subsidiary of UAO Nigeria PLC, Jos, Plateau State) was purchased from an open shop in Ekpoma, Edo State Nigeria.

All other chemicals (sodium bicarbonate used to dissolve indomethacin) were obtained from a chemical store in Ekpoma and the solution needed prepared in the physiology laboratory where the experiment was conducted and all other chemicals were of analytical grade.

The instruments (dissecting set, magnifying lens, weighing balance and centrifuge) used in this study were obtained from the Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State-Nigeria. Others such as plastic basket, Wattman No. 1 filter paper, orogastric cannula, plates for food and water, syringes, centrifuge bottles and hand gloves were obtained from a local store in and around Edo State, Nigeria.

Preparation of extract: The *Telfairia occidentalis* leaves were washed several times under running tap water. The fresh leaves were then blended and the grinded paste was mixed with some quantity of water and filtered. The filtrate was then allowed to sediment and the sedimented extract was left to dry in a water bath. This way, a powdery product was obtained from which aqueous extract was formed by dissolving appropriate grams in equal ml of distilled water to form the plant extract. This procedure is as previously described by Adisa *et al*., (2014).

Experimental animals: Male rats (N=30) weighing between 200±250g were procured from Animal Farm. The animals were fed standard diet (Grower's mash) and water given ad libitum. They were housed in six well-ventilated cages (each with a dimension of 45cm x 45cm x 45cm) under standard environmental conditions in a well-ventilated room under a 12/12 hours light/dark cycle and allowed two weeks of acclimatization.

Experimental grouping: After allowed two weeks (14 days) of acclimatization to the new environment, the animals were divided into groups as follows;

i. Group A (n = 5) serves as the control and received no treatment or ulcer induction.
ii. Group B serves as the test control 1 (n = 5). Ulcer was induced in this group with single oral administration of 40mg/kg indomethacin without treatment and scarified after four hours.
iii. Group C serves as the test control 2 (n = 5). Ulcer was induced in this group with single oral administration of 40mg/kg indomethacin without treatment for four hours and fed normal rat chow afterward and scarified after 72 hours (three days).
iv. Group D serves as the test (n = 5). The animals in this group received 100mg/kg of the aqueous leave extract of *Telfairia occidentalis* for 7 days without ulcer induction.
v. Group E serves as pre-treated test group (n = 5 rats). In this group of animals, ulcer was induced with 40mg/kg indomethacin after pre-treating the animal with 100mg/kg aqueous leave extract of *Telfairia occidentalis* daily for 7 days. Four hours after the induction of ulcer the animals were scarified.
vi. Group F serves as post-treated test (n = 5 rats). In this group of animals' ulcer was induced with 40mg/kg indomethacin and then four hours later was treated with 100mg/kg aqueous leave extract of *Telfairia occidentalis* daily for 7 days.

Experimental Procedure: Following two weeks of acclimatization, animals in each group received their corresponding treatments.

The doses chosen for indomethacin and aqueous leaf extract of *Telfairia occidentalis* were based on previous findings by Akpamu (2014) and Adisa *et al*. (2014) respectively. Indomethacin solutions was prepared by mixing indomethacin in required ml of sodium bicarbonate while aqueous leave extract of *Telfairia occidentalis* was prepared daily by mixing 1gm of the plant extract in 10ml of distilled water and the required quantity given to each rat per kg body weight. All suspension was given orally according to their body weight by oro-gastric iron cannula and was prepared within 15 to 30minutes before use.

Sample Collection: At the end of the treatments, animals were mildly anaesthetized with chloroform and the stomach harvested following standard laboratory procedures (Akpamu, 2014). The stomachs were obtained for the determination of ulcer indices and macroscopic evaluation.

Sample Analysis

a. Determination of gastric ulcer severity/ulcer score: Gross gastric lesions severity were measured as described by Wilhelmi and Menasse-Gdynia (1972) using the 0 to 5 scoring system. Severity factor 1 = 1 or 2 minutes, sporadic, punctuate lesion; 2 = several small lesion; 3 = one extensive lesion or multiple moderate sized lesions; 4 = several large lesions; 5 = several large lesions with stomach perforation.

The lesions score/ ulcer index (UI) for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor. The UI for each group was taken as the mean lesion score of all the rats in that group.

b. Determination of percentage ulcer inhibition: The percentage ulcer inhibition (%UI) of a given drug was calculated by the equation of Hano *et al.* (1976).

% UI = [(UI of ulcer control – UI of treated) / (UI of ulcer control)] × 100%.

c. Determination of marker of oxidative stress: The stomach was harvested and devoid of fat and accessory tissues. They were then patted dry with tissue paper and weighed and placed in a plain bottle containing homogenize buffer solution (phosphate buffer 1:10 w/v). The stomach was homogenized (grind using homogenizer machine) and the content (homogenate) centrifuged at 3000 rpm for 10 minutes to obtain the supernatant and stored at minus 20°C. This was used for the determination oxidative stress.

The protein concentration of the homogenate samples was determined by means of the Biuret method as described by Gornal *et al*., (1949). 5.0ml of blank Biuret reagent was prepared by dissolving CuSO4.5H2O crystals in 500mls of distilled water added to sample blank. These was mixed well and allowed to stand for 20 minutes at room temperature 25-27°C. Absorbance was read for test and standard against a blank at 540nm. The concentration of protein was calculated using:

$$\frac{\text{optical density for standard} \times \text{concentration of standard}}{\text{optical density for test sample}}.$$

MDA (an index of lipid peroxidation) was determined using the method of Buege and Aust (1978). l.0ml of the supernatant was added to 2ml of the Tricarboxylic acid-Thiobarbituric acid-Hydrochloric acid reagent. (TCA - TBA-HCL) reagent boiled at 100oC for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10 minutes. The supernatant was removed and the absorbance read at 532nm against a blank. MDA was then calculated using the molar extinction coefficient for MDA – TBA — Complex of 1.56 x 105M-1 CM-1.

e. Macroscopic (Gross) evaluation of gastric lesions: The stomachs were washed with saline water and examined for macroscopical mucosal lesions using magnifying lens. Ulcers of the gastric mucosa appear as inflammation and as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach rugus.

Macroscopic (Gross) Presentations: Macroscopic observations of the gastric mucosa were represented in pictures.

Statistical Analysis: The Statistical Package for Social Sciences (SPSS version 20) was used for data analysis. The one-way analysis of variance (ANOVA) was employed for data analysis and where applicable LSD was determined and confidential interval of $p \leq 0.05$ considered statistically significant. Results were presented as mean ± Standard deviation using suitable tables and charts.

RESULTS

Effect of aqueous leave extract of *Telfairia occidentalis* on ulcer indices in indomethacin induced gastric ulcers: Table 1 shows the effect of aqueous leave extract of *Telfairia occidentalis* on ulcer indices (ulcer severity, ulcer score and percentage ulcer inhibition potentials) in indomethacin induced gastric ulcers. It was the finding of this study that ulcer severity by indomethacin was highest in post-treatment with aqueous leave extract of *Telfairia occidentalis* (group F; 2.51±0.72) compared to pre-treatment (group E; 0.81±0.46) and this was followed by group B (2.26±0.97) and group C (2.01±0.01). The lowest ulcer severity was observed in the control and this was followed by pre-treatment with extract and then 7 days extract ingestion without ulcer induction (group D; 1.21±0.46). Compared to the control (Group A; 0.34±0.59), ulcer severity was significantly high ($p<0.05$) in groups B, C and F. Compared to group B, ulcer severity was significantly low ($p<0.05$) in groups A and E

Table 1.
The effect of aqueous leave extract of *Telfairia occidentalis* on ulcer indices in indomethacin induced gastric ulcers

Ulcer indices	Group A	Group B	Group C	Group D	Group E	Group F
Ulcer severity	0.34±0.59^{+}	2.26±0.97*	2.01±0.01*	1.21±0.46	0.81±0.46^{+}	2.51±0.72*
Ulcer score	0.34±0.39^{+}	10.26±4.06*	7.01±1.42*	3.01±1.88^{+}	1.01±0.72^{+}	10.01±2.82*
%UIP	0.00%	0.00%	32.72%	0.00%	91.25%	3.45%

*values are mean ± standard deviation; n = 5; * significant different at p<0.05 compared with control (group A); + significant different at p<0.05 compared with group B; Group A = Control; Group B = 4 hours ulcer induction without treatment; Group C = 72 hours ulcer induction without treatment; Group D = 7 days ingestion of extract without ulcer induction; Group E = 7 days pre-treatment with extract before 4hours ulcer induction; Group F = 7 days post-treatment with extract after 4 hours ulcer induction.*

Table 2.
The potential of aqueous leave extract of *Telfairia occidentalis* on indomethacin- induced oxidative stress**.**

Ulcer indices	Group A	Group B	Group C	Group D	Group E	Group F
Proteins	3.86±0.60^{+}	0.84±0.13*	1.79±0.61*$^{+}$	1.68±0.58*$^{+}$	2.28±0.75*$^{+}$	1.27±0.01*
MDA	0.55±0.07^{+}	4.45±0.86*	1.92±0.10^{+}	2.55±1.50	2.18±2.01^{+}	1.78±0.44^{+}

*values are mean ± standard deviation; n = 5; * significant different at p<0.05 compared with control (group A); + significant different at p<0.05 compared with group B; Group A = Control; Group B = 4 hours ulcer induction without treatment; Group C = 72 hours ulcer induction without treatment; Group D = 7 days ingestion of extract without ulcer induction; Group E = 7 days pre-treatment with extract before 4hours ulcer induction; Group F = 7 days post-treatment with extract after 4 hours ulcer induction*

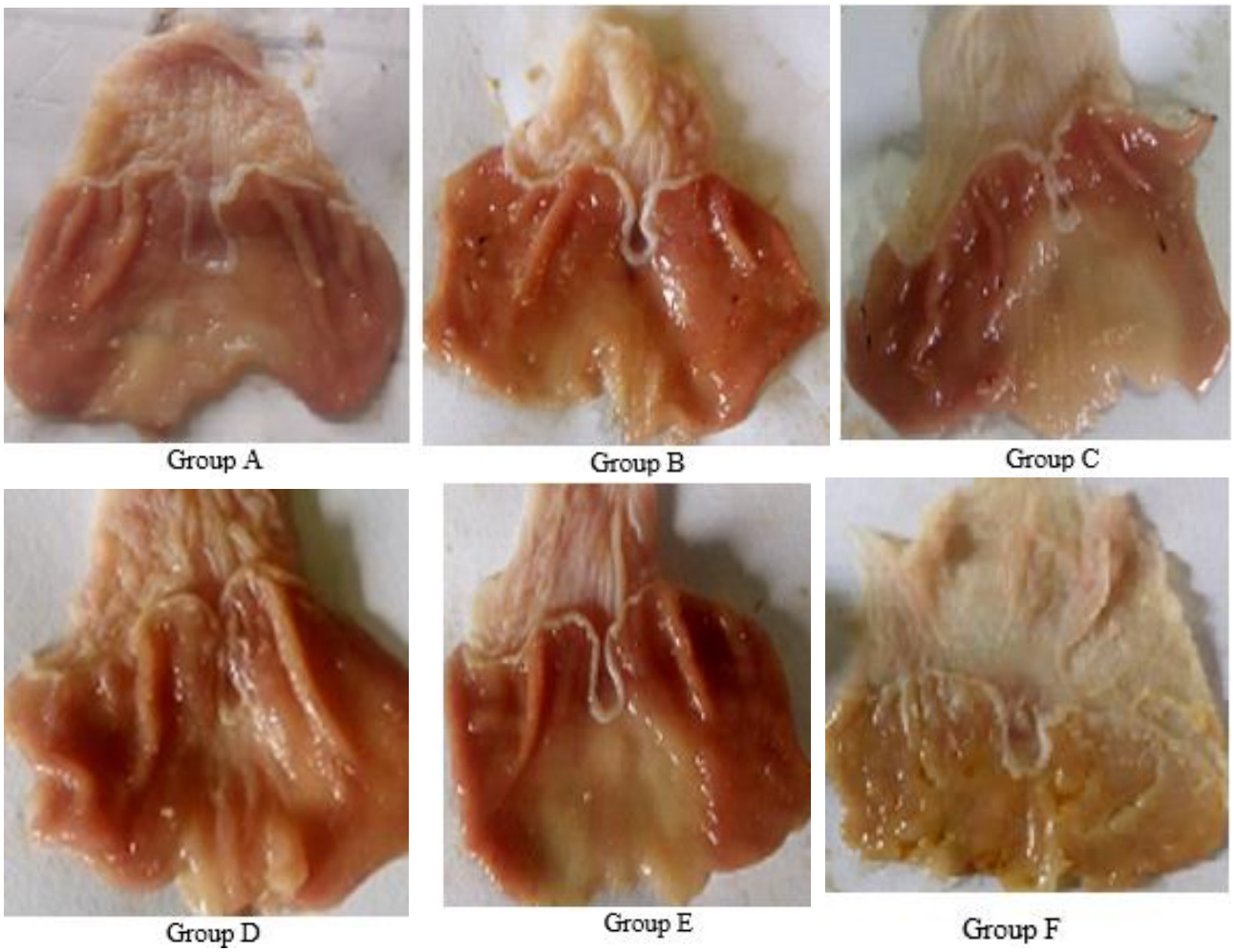


Plate 1.
Stomach macroscopic observation of the effect of the extract on indomethacin-induced gastric ulcers. **(**Group A = Control; Group B = 4 hours ulcer induction without treatment; Group C = 72 hours ulcer induction without treatment; Group D = 7 days ingestion of extract without ulcer induction; Group E = 7 days pre-treatment with extract before 4hours ulcer induction; Group F = 7 days post-treatment with extract after 4 hours ulcer induction).

Ulcer score was highest in group B (10.26±4.06) and this was followed by group F (10.00±2.83) and then group C (7.00±1.41) and these were significant (p<0.05) compared to the control (group A; 0.34±0.39). Pre-treatment with the extract before ulcer induction (1.01±0.72) resulted in a significant lower (p<0.05) ulcer score compared to post-treatment (10.01±2.84). Ingestion of the extract without ulcer induction was observed to induced minimal ulcer score (3.01±1.88) that was not significantly higher (p>0.05) than the control (0.34±0.39).

The ulcer inhibition potential of aqueous leave extract of *Telfairia occidentalis* on indomethacin induced gastric ulcers as presented showed that pre-treatment with the extract resulted in a 91.25% protection potential. Whereas, the post-treatment resulted in a 3.45% healing capacity. Interestingly, there was a 32.72% self-healing capacity when indomethacin induced ulcer is left untreated for 72 hours (3 days).

The potential of aqueous leave extract of *Telfairia occidentalis* on indomethacin-induced oxidative stress (MDA and protein): Table 2 shows the impact of aqueous leave extract of *Telfairia occidentalis* on indomethacin-induced oxidative stress. Compared to the control (3.86±0.60), protein concentration was significantly reduced in all other groups. However, compared to group B (0.84±0.13), protein concentration was significantly higher in groups A (3.86±0.60), C (1.79±0.61), D (1.68±0.58) and E (2.28±0.75).

MDA concentration was highest in group B (4.45±0.86) and this was followed by group D (2.55±1.50) and then Group E (2.18±2.01). On the other hand, MDA concentration was lowest in the control (Group A; 0.55±0.07) and this was followed by group F (1.78±0.44) and the group C (1.92±0.10). Statistically, MDA was significantly higher ($p<0.05$) in groups B (4.45±0.86) compared to control (0.55±0.07) but significantly lower ($p<0.05$) in group A (0.55±0.07), C (1.92±0.10), E (2.18±2.01) and F (1.78±0.44) compared to group B (4.45±0.86).

Gastric macroscopic effect of treatments with aqueous leave extract of *Telfairia occidentalis* on indomethacin-induced gastric ulcers: Plate 1 indicate the macroscopic observations of the protective and healing potentials of aqueous leave extract of *Telfairia occidentalis* on indomethacin-induced gastric ulcers. Note the mild reduced mucus in group A. Group B presented thick mucosal with several perforated mucosa and mildly spread mucosa inflammation. Group C presented several elongated mucosa perforations with thick mucosa. Group D presented thick mucosal with mildly localized inflammations. Pre-treatment with extract (group E) presented widely spread severe hemorrhagic inflammation with thin mucosa, while the post-treated, Group F presented several perforated ulcerations that are healing with thick mucosa.

DISCUSSION

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is considered to be the major risk factor in gastric ulcers. In fact, the deleterious effects of Indomethacin on different diseases are well known and it induces gastric ulcers both in humans and experimental animals (Ilahi *et al*., 2006). This study also confirmed the ulcerogenic potential of indomethacin on gastric mucosa as observed in group B. The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the reepithelization of the ulcer crater (Levi *et al*. 1990).

There has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as peptic ulcer (Pratt, 1992). In the present study, indomethacin caused damage on the glandular mucosa with a high ulcer index (see table 1), increased MDA level (table 2), alterations of macroscopic structure of gastric mucosa (figure 1). In contrast to this observed effect of indomethacin, pre-treatment with aqueous leave extract of *Telfairia occidentalis* at the dose of 100 mg/kg body weights (see group E) significantly decreased ($P<.05$) the ulcer index (table 1), MDA level (table 2) and restored the gastric mucosa architecture (figure 1) with indomethacin-induce ulcer inhibition potential of 91.25%. On the other hand, although post-treatment with aqueous leave extract of *Telfairia occidentalis* at the dose of 100 mg/kg body weight (group F) reduced gastric MDA level (table 2), there were distorted macroscopic damage in the mucosa and a weak ulcer inhibition of 3.45% (table 1). This indicates therefore that aqueous leave extract of *Telfairia occidentalis* at the dose of 100 mg/kg body weights may slow ulcer healing considering the 31.71% ulcer healing potential of group C; that was untreated for 72 hour. The finding of this study is consistent with that of Airaodion *et al*., (2019), that *Telfairia occidentalis* was able to remedy the effect of ethanol by regulating the oxidative stress biomarkers, thus possesses therapeutic effect against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress. This also appeared to be in line with the finding of Oboh (2005), while Adejuwon *et al*., (2014) reported that *Telfairia occidentalis* at lower doses could significantly improve oxidative stress related pathological changes in brain of irradiated rats.

Although the protective mechanism of aqueous leave extract of *Telfairia occidentalis* against indomethacin induced gastric damage was not investigated in this study, it is however believed to be associated with the components of the extract. This assertion is based on the fact that *Telfairia occidentalis* is rich in minerals, antioxidants, vitamins and essential oils (Oboh *et al*., 2004, 2006; Nwanna and Oboh, 2007; Adaramoye *et al*., 2007; Emeka and Obidoa, 2009; Kayode *et al*., 2009; Kayode *et al*., 2010). Thus, the gastro protective effect with pre-treatment may be linked to its antioxidants and vitamins component. This may explain the reduced MDA levels in groups D, E and F compared to group B. Vitamins; for example vitamin C has been reported by Akpamu (2014) to attenuates the deleterious effect of indomethacin on ulcer and this was said to be due to its anti-oxidant activity by mechanism involving preservation of gastric microcirculation, attenuation of lipid peroxidation and release of pro-inflammatory cytokines (Konturek *et al*., 2006). Administration of both indomethacin and vitamin C induce less gastric mucosal damage due to the increase in expression and activity of hemeoxygenase-1(HO-1). HO-1 plays an important role in gastric protection against indomethacin, by making cells more resistant to apoptotic death (Zhu *et al*., 2000). Therefore, the observed ulcer preventive and weak ulcer curative activity of the aqueous leave extract of *Telfairia occidentalis* may be partially due to its relative antioxidant activity and its phyto-chemical constituents. The assertion of its phyto-constituents is based on the fact that phyto-constituents like flavonoids, tannins, terpenoids, and Saponin have been reported in several anti-ulcer literatures as possible gastro protective agents (Pandian *et al*. (2000).

In conclusion, the analyses of ulcer indices and macroscopic examinations showed that indomethacin is ulcerogenic and that aqueous leave extract of *Telfairia occidentalis* increased gastric protein level, reduced MDA level and restored the macroscopic structure of gastric mucosa. The best results were observed in rats pre-treated with aqueous leave extract of *Telfairia occidentalis* where, ulcer indices and stomach injury were almost restored to the pre-ulceration states. This indicates therefore that aqueous leave extract of *Telfairia occidentalis* possesses more of a protective effect than a healing potential.

Acknowledgement: The authors wish to acknowledge the technical support of Akpamu U. and P.A Ogarah

REFERENCES

Adaramoye, O.A., J. Achem, O.O. Akintayo and Fafunso, (2007). Hypolipidemic effect of *Telfairia occidentalis* (fluted pumpkin) in rats fed a cholesterol-rich diet. J. Med. Food; 10: 330-336.

Adejuwon S. A., Imosemi I.O., Ebokaiwe P.A., Omirinde J.O. and Adenipekun A.A., 2014. Protective role of *Telfairia occidentalis* in irradiation-induced oxidative stress in rat brain. Int. J. Biol. Chem. Sci. 8(3): 843-853.

Adisa, W.A., Nwankwo, A.A., Eidangbe, A.P., Uwuigbe, M., Olugbenga, M.A. Gastro-protective effects of aqueous extract of *Telfairia occidentalis* in indomethacin induced gastric ulcer. International Journal of Herbs and Pharmacological Research, 2019, 8(1): 9- 19.

Adisa W.A., Olugbenga M.A., Bankole J.K., Uwuigbe M., Eidangbe A.P. (2018). Protective and therapeutic potential of *Telfairia occidentalis* on blood glucose level in alloxane induced diabetes mellitus in adult male rats. International Journal of Herbs and Pharmacological Research; 7(1): 31-39.

Adisa W.A., Okhiai O., Bankole J.K., Iyamu O.A., Aigbe O. (2014). Testicular Damage in *Telfairia occidentalis* Extract Treated Wista Rats. American Journal of Medical and Biological Research; 2(2): 37-45.

Akindele A.J., Ajao M.Y., Aigbe F.R., Enumah U.S. (2013). Effects of *Telfairia occidentalis* (fluted pumpkin; Cucurbitaceae) in mouse models of convulsion, muscle relaxation, and depression. J Med Food; 16 (9): 810-816.

Airaodion1 A.I., Ogbuagu E.O., Ekenjoku J.A., Ogbuagu U., and Airaodion E.O. (2019). Therapeutic Effect of Methanolic Extract of *Telfairia occidentalis* Leaves against Acute Ethanol-Induced Oxidative Stress in Wistar Rats. International Journal of Bio-Science and Bio-Technology (IJBSBT); 11(7): 179-189.

Akoroda, M.O. (1990). Ethnobotany of *Telfairia occidentalis* (cucurbitaceae) among Igbos of Nigeria. Econ. Bot; 44: 2939.

Akoroda, M.O., 1990. Seed production and breeding potential of the fluted pumpkin, *Telfairia occidentalis*. Euphytica; 49: 25-32.

Akpamu, U. (2014). Combined effect of testosterone and vitamin C on indomethacin induced peptic ulcers in female Sprague Dawley rats. A project submitted to the Department of Human Physiology, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, in partial fulfillment for the award of a Master of Science Degree in Human Physiology, 2014.

Bansala V. K., Goel R.K. (2012). Gastroprotective effect of Acacia nilotica young seedless pod extract: Role of polyphenolic constituents. Asian Pacific Journal of Tropical Medicine; 5 (7):523-528.

Bourdet, L. David, J. B., Pritchard, D. and Thakker, R. (2005). Differential substrate and inhibitory activities of ranitidine and famotidine toward human organic cation transporter 1 (hOCT1; SLC22A1), hOCT2 (SLC22A2), and hOCT3 (SLC22A3). Journal of pharmacology and experimental therapeutics; 315(3):1288-1297.

Bagchi, M., Milnes, M., Williams, C., Balmoori, J., Ye, X., Stohs, S. and Bagchi, D. (1999). Acute and chronic stressinduced oxidative gastrointestinal injury in rats and the protective ability of a novel grape seed proanthocyanidian extract. Nutr. Res.; 19: 1189.

Calam O. and Baron J.H. (2001). Pathophysiology of duodenal and gastric ulcer and gastric cancer. BMJ; 323:980.

Chaturvedi, A., Kumar, M., Hawani, G., Chaturvedi, H. and Goel, R.K. (2007). Effect of ethanoic extract of Eugenia jamolana seeds on gastric ulceration and secretion in rats. Indian J. Physiol. Pharmacol; 51: 131-140.

Emeka, E.J.I. and Obidoa, O. (2009). Some biochemical, haematological and histological responses to a long term consumption of *Telfairia occidentalis*-supplemented diet in rats. Pak. J. Nutr; 8: 1199-1203.

FAO (1989). Some medicinal plants of Africa and Latin America. FAO Forestry Paper, 67.

Fisher A.A. and Le Couteur D.G. (2012). Nephrotoxicity and Hepatotoxicity of Histamine H2 Receptor Antagonists. Springer Link.

Gbile, Z.O. (1986). Ethnobotany, Taxonomy and Conservation of Medicinal Plants. In: The State of Medicinal Plants Research in Nigeria, Sofowora, A. (Ed.). University of Ibadan Press, Ibadan, Nigeria.

Gornal AG, Bardwil GS, David MM. 1949. Determination of serum proteins by the means of Biuret reactions. J Biol Chem. 177:751–766.

Hano, J., Bugajski, J. and Danek, L. (1976). Effect of adrenergic blockade on gastric secretion altered by catecholamine's in rats. Arch. Immunol. Ther. Exp. (Warsz), 24(4):507- 524.

Ilahi, M, Khan, J, Inayat, Q., Abidi, T.S. (2006). Histological changes in parts of foregut of rat after indomethacin administration. J Ayub Med Coll Abottabad; 18(3): 29-32.

Kayode, A.A.A. and Kayode, O.T. (2011). Some Medicinal Values of *Telfairia occidentalis*: A Review. American Journal of Biochemistry and Molecular Biology; 1: 30-38.

Kayode, A.A.A., Kayode, O.T. and Odetola, A.A. (2010). *Telfairia occidentalis* ameliorates oxidative brain damage in malnorished rats. Int. J. Biol. Chem; 4: 10-18.

Kayode, O.T., Kayode, A.A. and Odetola, A.A. (2009). Therapeutic effect of *Telfairia occidentalis* on protein energy malnutrition-induced liver damage. Res. J. Med. Plant; 3: 80-92.

Jung, U.P., JiHoon, K., Aziz, A., Ahtesham, H., Jin Sook, C. and Young, I. L. (2019). Gastroprotective effects of Plants Extracts on Gastric Mucosal Injury in Experimental Sprague-Dawley Rats. BioMed Research International; Article ID 8759708 11 pages.

Kim, J.W. (2008). NSAID-induced gastroenterpathy. Korean J. Gastroenterol.; 52(3):134-141.

Konturek, C.P., Kania, J., Hahn, G.E. and Konturek, W.J. (2006). Ascorbic acid attenuates aspirin-induced gastric damage: role of inducible nitric oxide synthase. Journal of Physiology and Pharmacology; 57(5): 125 – 136.

Kurekci, C., Bishop-Hurley, S.L., Vercoe, P.E., Durmic, Z., Al Jassim, R.A., McSweeney, C.S. (2012). Screening of Australian plants for antimicrobial activity against Campylobacter jejuni. Phytother Res; 26:186–90.

Kuku, A., Etti, U. and Ibironke, I.S. (2014). Processing of fluted pumpkin seeds, *Telfairia occidentalis* (hook f) as it affects growth performance and nutrient metabolism in rats. African journal of food agriculture nutrition and development; 14 (5).

Lanas, A, Carrera-Lasfuentes, P, Garcıa Rodrguez, L.A., *et al*. (2015). Outcomes of peptic ulcer bleeding following treatment with proton pump inhibitors in routine clinical practice: 935 patients with high- or low-risk stigmata. Scand J Gastroenterol; 49: 1181–90.

Lanas, A. and Chan, F.K. (2017). Peptic ulcer disease. Lancet; 390:613-24

Leontiadis, G.I., Molloy-Bland, M., Moayyedi, P., Howden, C.W. (2013). Effect of co-morbidity on mortality in patients with peptic ulcer bleeding: systematic review and meta-analysis. Am J Gastroenterol; 108: 331–45.

Levi, S., Goodlad, R.A. and Lee, C.Y. (1990). Inhibitory effect of NSAIDs on mucosal cell proliferation associated with gastric ulcer healing. Lancet 336(8719):840-843.

Miami, H., Kautianen, H., Virta, L.J. and Farkkila, M.A. (2016). Increased short- and long-term mortality in 8146 hospitalised peptic ulcer patients. Alimentary Pharmacology and Therapeutics; 44:234.

Malyshenko, O.S., E.I. Beloborodova, A.M. Vavilov, G.V. Lomivorotova and V.I. Kasperskaia, 2005.Impact of age and type of behavior on the course of ulcer disease. Ter. Arkh; 77: 28-31.

Morufu E. B., Nwachukwu, D., Onwe, P.E., and Folawiyo, M.A. (2014). Gastric acid anti-secretory effects of aqueous leaf extract of Nauclealatifolia (Rubiaceae) in rats. The Journal of Phytopharmacology; 3(6): 389-394.

Nwanna, E.E. and Oboh, G (2007). Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted Pumpkin) leaves on acetaminophen induced liver damage. Pak. J. Biol. Sci; 10: 2682-2687.

Oboh, G. and Akindahunsi, A.A. (2004). Change in the Ascorbic Acid, Total Phenol and Antioxidant Activity of Some Sun-Dried Green Leafy Vegetables in Nigeria. Nutrition and Health; 18: 29-36.

Oboh, G., 2005. Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (*Telfairia occidentalis*) leaves against garlic-induced oxidative stress. J. Med. Food; 8: 560-563.

Oboh, G., Nwanna, E.E. and , C.A. (2006). Antioxidant and antimicrobial properties of *Telfairia occidentalis* (Fluted pumpkin) leaf extracts. J. Pharmacol. Toxicol., 1: 167-175.

Pandian RS, Anuradha CV, Viswanathan P. (2002).Gastroprotective effect of fenugreek seeds (Trigonellafoenumgraecum) on experimental gastric ulcer in rats. J Ethnopharmacol.81:393–7.

Pohle, T., Brzozowski, T., Becker, J.C., Van Der Voort, I.R., Markman, A. and Konturek, S.J. (2001). Role of reactive oxygen metabolites in aspirin-induced gastric damage in humans: gastroprotection by vitamin C. Aliment Pharmacol. Ther.; 15: 677–687.

RibeiroFilho, E. ; Paiva, P. C. de A. ; Barcelos, A. F. ; Rezende, C. A. P. ; Cardoso, R. M. ; Banys, V. L., 2000. The effect of coffee hulls on the performance of Holstein-zebu steers during the growing period. Ciencia e Agrotecnologia; 24 (1): 225-232.

Rates, S.M.K. (2001). Plants as source of drugs. Toxicon, 39(5): 603-613.

Repetto, M.G.1. and Llesuy, S.F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res; 35(5):523-34.

Schmeda-Hirschmanna G. and Yesiladab E. (2005). Traditional medicine and gastroprotective crude drugs. Journal of Ethnopharmacology; 100(1–2): 61-66.

Tortora, G.J. and Grabowski, S.R. (2003). Principles of Anatomy and Physiology.10th ed. John Wiley & Sons, Inc, NJ, p.889.

Toyin, M. S., Isiaka, A. A., Sikiru, A. B., Olusegun, A.A., Opeyemi, K. O., Olanrewaju, A.A.(2013). Blood glucoselowering effect of *Telfairia occidentalis*: A preliminary study on the underlying mechanism and responses. Biokemistri An International Journal of the Nigerian Society for Experimental Biology; 25 (3): 133–139.

Wedemeyer, R.-S., and Blume, H. (2014). Pharmacokinetic drug interaction profiles of proton pump inhibitors: an update. Drug Safety; 37(4): 201–211.

Whittle J. R.B. (2003). Gastrointestinal effects of nonsteroidal anti- inflammatory drugs. Fundamental & Clinical Pharmacology; 17(3): 301-313.

Wilhelmi, G. and Menasse- Gdynia, R. (1972). Pharmacology; 8: 321-328. In: Sadik, S.A.E. (1984).

Zhu GH, Wong BC, Slosberg ED, Eggo MC, Ching CK, Yuen ST, Lai KC, Soh JW, and Weinstein IB, Lam SK. (2000): Overexpression suppresses indomethacin-induced apoptosis in gastric epithelial cells. Gastroenterology. 118: 507-514.

Niger. J. Physiol. Sci. 35 (December 2020): 203 - 208
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Vinpocetine Prevents Haloperidol-Induced Cognitive and Working Memory Deficits Through Attenuation of Oxidative and Nitrosative Stress in Mice

***Ishola I.O., Isaac A.I. and Adeyemi O.O**

Department of Pharmacology, College of Medicine, University of Lagos. Lagos, Nigeria.

Summary: There is a strong interplay between central mechanisms regulating emotional and cataleptic states similar to that observed in Parkinson's disease (PD). Several drugs including haloperidol have been implicated in the aetiology of PD. However, prolong treatment of PD patients with current available drugs induced dyskinesia, hence, the need for better treatment options. Vinpocetine, a derivative of the alkaloid vincamine used as a dietary supplement to enhance cognition and memory. Several studies have reported therapeutic benefit of vinpocetine in various disease conditions. Thus, this study sought to investigate the protective effect of vinpocetine against haloperidol-induced catalepsy in mice. Vinpocetine (5, 10 or 20 mg/kg, p.o.) was administered 1 h after haloperidol injection for 21 consecutive days. Effect on motor coordination, depressive-like behaviour and working memory were assessed with rotarod, forced swim test (FST) and Y-maze test (YMT), respectively. Brains were collected on day 21 for biochemical estimation of nitrosative and oxidative stress parameters. Vinpocetine (10 or 20 mg/kg, p.o.) significantly reversed haloperidol-induced motor deficit in rotarod test and open field test and reduced the duration of catalepsy during acute and subchronic catalepsy tests as compared to trihexylphenidyl but failed to reverse haloperidol-induced memory deficit in the Y-maze test. Haloperidol-induced increase in malondialdehyde and nitrite generation as well as deficits in antioxidant enzymes activities were attenuated by subchronic administration of vinpocetine. These findings suggest that vinpocetine protects against haloperidol-induced catalepsy and motor deficits through attenuation of oxidative/nitrosative stress.

Keywords*: catalepsy; haloperidol; memory; oxidative stress; Parkinsonism; Y-maze test*

**Address for correspondence: oishola@cmul.edu.ng; Tel: +234-8033018908*

Manuscript received: March 2020; Revised version accepted: December, 2020

INTRODUCTION

Parkinson's disease (PD) is the second most common age-related neurodegenerative progressive disorder and affects basal ganglia circuitry. It is characterized by extensive dopamine neuron degeneration in the substantia nigra pars compacta (SNpc) exerting an impact on the striatum as well as on target cortical and limbic areas (Björklund and Dunnett, 2007). It was estimated that over five million people worldwide have PD (Fahn and Przedborski, 2008). There is substantial geographic and ethnic variability in the prevalence and incidence in Africa with generally lower rate reported (Okubadejo *et al*., 2006). Most obvious symptoms early in the course of the disease are movement-related, such as resting tremor, muscular rigidity, bradykinesia and postural imbalance (Lotharius and Brundin, 2002; Olanow, 2007). The mechanism for dopaminergic neuronal degeneration in PD is not completely clear, but it is believed that oxidative and nitrosative stress play important role during the disease pathogenesis. This notion is supported by findings on an increase of several indices of oxidative and nitrosative stress (overproduction of nitric oxide (·NO), often complicated by simultaneous production of superoxide anions, which results in the formation of peroxynitrite and other reactive nitrogen species) in PD patients (Khan *et al*., 2012; Nakamura and Lipton, 2011; Polydoro *et al*., 2004). The selective dopaminergic degeneration in PD suggests that generation of oxidative stress associated with dopamine metabolism is an important contributor (Tsang and Chung, 2009). In addition, postmortem studies of brains from PD victims suggest that oxidative stress plays an important role in the degeneration of dopaminergic neurons in the SNpc (Fahn and Cohen, 1992).

The discovery that administration of haloperidol to rodents led to a transient Parkinsonian-like state was rapidly followed by the key discovery that these symptoms were reversed by the administration of L-DOPA (Duty and Jenner, 2011; Knol *et al*., 2012). Haloperidol works by antagonizing dopamine D_2 and, to a lesser extent, D_1 receptors in medium spiny neurons that comprise the indirect and direct pathways of the motor circuit respectively. The resultant block of striatal dopamine transmission results in abnormal downstream firing within the basal ganglia circuits that is manifest as symptoms of muscle rigidity and catalepsy within 60 min of haloperidol injection (Duty and Jenner, 2011). Moreover, acute administration of haloperidol has been shown to reduce the striatal content of dopamine, noradrenaline and 5-HT (Kulkarni *et al*., 2009). Haloperidol induces a six-fold increase in levels of reactive oxygen species (ROS), which are generated from mitochondria but not from the

metabolism of catecholamines by monoamine oxidases (Sagara, 1998).

Vinpocetine (marketed as Cognitol®), a derivative of the alkaloid vincamine isolated from periwinkle plant, has been clinically used in many countries for treatment of cerebrovascular disorders such as stroke and dementia for more than 30 years. Currently, vinpocetine is also available in the market as a dietary supplement to enhance cognition and memory. Due to its excellent safety profile, increasing efforts have been put into exploring the novel therapeutic effects and mechanism of actions of vinpocetine in various cell types and disease models. (Zhang *et al*., 2018). Earlier studies on anti-inflammatory (Zhang *et al*., 2014; Wu *et al*., 2017), vascular remodeling and anti-atherosclerosis (Cai *et al*., 2012) action of vinpocetine have been reported. We earlier showed that vinpocetine prevented paraquat-induced motor deficits, memory impairment, oxidative stress and neuroinflammation through enhancement of antioxidant defense system and inhibition of neuroinflammatory cytokine in mice (Ishola *et al*., 2018). Thus, this study sought to evaluate the protective effect of vinpocetine on haloperidol-induced Parkinsonism in mice. Findings from this study may facilitate the repositioning of vinpocetine in the treatment of PD in humans.

MATERIALS AND METHODS

Drugs and chemicals: Haloperidol 4-[4-(4-chlorophenyl)-4-hydroxypiperidinol]-4-fluorobutyrophenone, sodium hydroxide, Triton x-100, sodium chloride (NaCl), sodium nitrate (NaNO2), sulphanilamide, napthaylamine diamine dihydrochloric, bovine serum albumin (BSA), 5, 5-dithiobis (2 nitrobenzoic acid) (DTNB), Folin–Ciocalteu's, hydrochloric acid, trichloroacetic acid and 2-thiobabituric acid (TBA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Vinpocetine and trihexylphenidyl hydrochloride (Himadri Specialty chemical Ltd, Bengal, India).

Laboratory animals: Adult Swiss male albino mice (15-21g) used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were fed with rodent chow and had free access to drinking water. The experimental animals were generally maintained at 26±2°C and a relative humidity of about 55±5%.

Treatment regimen: Fifty-six mice were randomly divided into seven groups (n=8) and were orally treated as follows for 21 consecutive days; group I – vehicle, normal saline treated (normal control; 10ml/kg), group II – vinpocetine (20mg/kg), group III –vehicle (10ml/kg) + haloperidol (1mg/kg, i.p.), normal saline treated (PD model control; 10ml/kg), groups IV-VI - vinpocetine (5, 10 or 20 mg/kg, respectively) and group VII - trihexylphenidyl HCl (0.1mg/kg) for 21 consecutive days. Sixty minutes post-treatment animals in groups III-VII were given haloperidol (1mg/kg; i.p.) for 21 consecutive days.

Rotarod test: Mice were subjected to rotarod test to evaluate the effect of haloperidol and vinpocetine on muscle coordination. The apparatus consists of a horizontal metal rod (6 cm in diameter) attached to a motor with fixed speed. The rod is divided into five sections by a partition disc (10.5 cm in diameter). The rod was placed at a height of 50 cm to discourage the animals from jumping from the rotating rod. The animals were trained on the rotarod at a fixed speed of 20 rpm until they could remain on the apparatus for 300 s without falling 24 h before the experiments. Animals were subjected to rotarod test, one hour after drug or vehicle treatments on days 1, 4 and 11. The latency of falling within 5 min of test was recorded (Ishola *et al*., 2018).

Open Field test: To test effect of treatments on motor coordination and anxiety like behaviour, the open field test (OFT) was carried out. The apparatus was made of wood 50cm in length, 50cm in width and 25cm in height. The plain floor of the box was divided into 16 squares (8cm by 8cm). One-hour post oral administration, the mouse was gently placed at the centre of the open field apparatus and was allowed to acclimatize for 1 min, then the spontaneous activities were recorded for 5 mins. The following parameters were recorded: total number of crossing (number of times a mouse crosses from one square to another with all the four paws), total number of centre crossing (number of times a mouse enter the centre square where it was placed initially) (Ishola *et al*., 2014).

Y-maze test: The test relies on the innate tendency of mice to explore a novel environment to assess spatial recognition (Sarter *et al*., 1988). Animals were placed at the centre of the Y-maze facing the south arm 'B' and allowed to explore the maze freely for a period of 5 min. The number and the sequence of arm entries were observed. An arm entry was scored when all four paws were in the arm. Alternation behavior was defined as consecutive entries into all three arms (i.e., ABC, CAB, or BCA but not BAB). The percentage of spontaneous alternation was measured as an index of working memory by calculating the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100, i.e., % alternation [(number of alternations) / (total number of arm entries − 2)] − 100. The total number of arm entries was measured as an index of locomotor activity (Adedeji *et al*., 2014).

Catalepsy bar test (acute and chronic assays): Catalepsy bar test is widely used to measure the failure to correct an imposed posture resulting from muscular rigidity. It consists of placing a mouse forepaw on an elevated bar (4cm × 4cm) with the hind paws remaining on the floor. The time taken for the mouse to remove its paw from the bar, is an index of the intensity of catalepsy. A cataleptic mouse will continue to hold onto the bar for a prolonged period of time while a normal mouse will change its position within seconds (Adedeji *et al*., 2014) Hence, the ability of vinpocetine to ameliorate haloperidol-induced catalepsy was assessed using the bar test using the method of Adedeji *et al*. (2014). The bar is made up of wooden square bar (4cm × 4cm). Mice were gently positioned, placing their forelimbs on the bar and their hind limbs on the floor of the apparatus. The duration of catalepsy (animal is considered cataleptic if it maintained the imposed posture for at least 20 s), was recorded at 15, 30, 45, 90, and 120min for acute study on day 1. The endpoint of catalepsy was considered to occur when both forepaws were removed from the bar. A cut-off

time of 300 s was applied, all the observations were made in a quiet room. For the chronic study, catalepsy bar test was repeated on days 5, 9, 13, 17 and 21, respectively.

Brain perfusion and isolation: One hour after last treatment on day 21, mice were anaesthesized with chloral hydrate (300 mg/kg, i.p.) and perfused intracardially with chilled normal saline and brain was rapidly dissected out and rinsed in chilled phosphate buffer saline (0.03M) on ice. A 10% (w/v) homogenate of brain samples (0.03M sodium phosphate buffer, pH 7.4) was prepared by using an homogenizer at a speed of 9500 rpm. The homogenized tissue preparation was used to measure oxidative / nitrosative stress markers (Ishola *et al*., 2018).

Determination of oxidative/nitrosative stress markers: MDA an index of lipid peroxidation was determined using the method of Buege and Aust (1978), reduced glutathione (GSH), as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). Catalase activity (CAT) was determined according to Sinha (1972). Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Ishola *et al*. (2019). Nitrite was estimated in the brain using the Greiss reagent and served as an indicator of nitric oxide production (Green *et al*., 1982). Protein concentration was estimated using Lowry method (Upreti *et al*., 2012).

Statistical analysis

Values are expressed as mean ± SEM (n=8) and data analyzed using one- or two-way analysis of variance (ANOVA) followed by the Tukey's post-hoc multiple comparison tests. Level of statistical significance is $p<0.05$.

RESULTS

Rotarod performance test: Two way ANOVA revealed significant effect of treatments [F(6,105)=22.41,P<0.0001] and interaction between haloperidol and treatments [F(12,105)=3.67,P=0.0001]. Figure 1 showed that the intraperitoneal injection of haloperidol induced loss of muscle coordination on days 1, 4 and 11 when compared with vehicle treated control. However, co-administration of vinpocetine (5, 10 or 20 mg/kg) reversed haloperidol-induced motor deficit in mice.

Open Field Test: The administration of haloperidol reduced the number of crosses made by the mice when compared to the vehicle-control group (Table 1). However, vinpocetine (10mg/kg and 20mg/kg) treatment significantly ($p<0.01$) increased the number of crosses when compared to vehicle-treated haloperidol control on days 12, 15 and 18. Two way ANOVA showed significant effect of treatments (F(6,105)=17.21, $P<0.01$).

Y-maze test: The effect of vinpocetine on working memory was investigated in the spontaneous alternation behaviour Y-maze test. Haloperidol injection caused significant ($p<0.001$) decrease in relative proportion of spontaneous alternation behavior when compared to normal-vehicle treated control on days 6 and 9. However, the decrease in the relative proportion of spontaneous alternation behavior induced by haloperidol was reversed by vinpocetine (5, 10 and 20mg/kg) administration. Two way ANOVA revealed significant effect of treatments [F(6,105)=3.59,P<0.0001] and interaction between haloperidol and treatments [F(12,105)=2.68,P=0.0001] (Fig. 2).

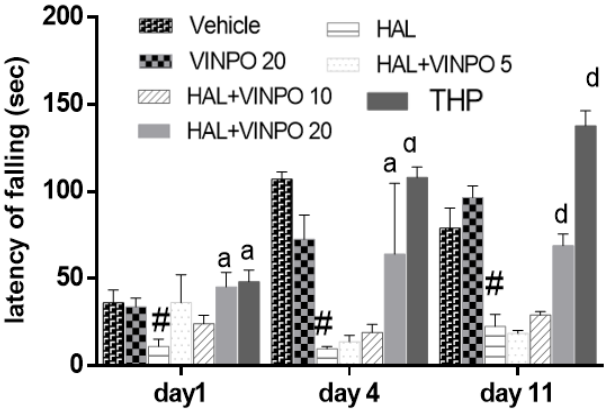


Figure 1:
Effect of vinpocetine on haloperidol-induced motor coordination on days 1, 4 and 10 in rotarod test. Values are expressed as mean±SEM (n=8). Significant levels of significance analysed by two-way ANOVA followed by Turkey post hoc multiple comparison tests. $^{\#}P<0.05$ versus vehicle 10ml/kg normal untreated mice; $^{a}P<0.05$, $^{d}P<0.0001$ versus HAL treated mice group. Key: VINPO = Vinpocetine, HAL = Haloperidol, THP = Trihexylphenidyl HCl.

Table 1:
Effect of haloperidol and vinpocetine on locomotor activity in open field test

Treatment	Dose (mg/kg)	day 12	day 15	day 18
vehicle	10ml/kg	67.40 ±2.06	61.80 ±2.59	62.00 ±6.76
vinpocetine	20	51.20 ±4.39	64.20 ±2.33	79.00 ±2.12
haloperidol	1	48.40 ±12.17$^{\#}$	25.80 ±4.11$^{\#}$	20.80 ±1.77$^{\#}$
vin+HAL	5+1	35.20 ±9.47	32.40 ±5.39	35.80 ±1.35
vin+HAL	10+1	62.40 ±12.87	53.60 ±3.66	54.00 ±2.00^{a}
vin+HAL	20+1	91.20 ±20.05^{b}	64.60 ±8.33^{b}	71.00 ±5.73^{d}
THP+HAL	0.1+1	64.40 ±4.13	75.60 ±1.24^{d}	71.00 ±1.87^{d}

Results are expressed as mean ± SEM (n = 8). $^{\#}P<0.05$ versus vehicle 10ml/kg normal untreated mice; $^{a}P<0.05$,$^{b}P<0.01$, $^{d}P<0.0001$ versus HAL-pretreated mice group. Statistical level of significance analysis by two way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Key: VINPO = Vinpocetine, HAL = Haloperidol, THP = Trihexylphenidyl HCl.

Acute Haloperidol-induced catalepsy test: Oral administration of vinpocetine reversed the induced cataleptic effect of haloperidol in a dose dependent manner. Post hoc analysis showed that the intraperitoneal injection of haloperidol induced significant ($p<0.01$) cataleptic behaviour that peaked at 2 h post-treatment. In contrast, oral administration of vinpocetine produced significant and time

course reduction in haloperidol-induced cataleptic behaviour [F (6,105) =107.10, P< 0.0001] (Table 2).

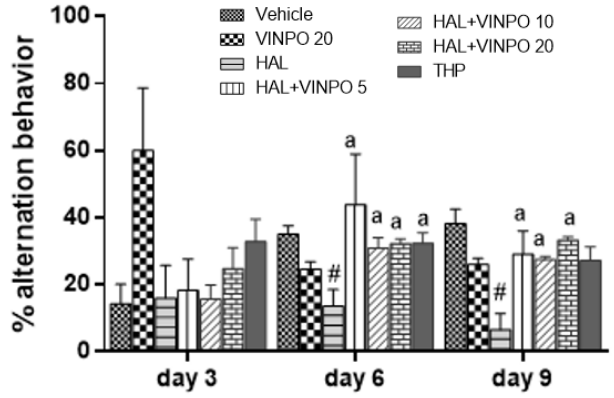


Figure 2:
Effect of vinpocetine treatment on haloperidol-induced working memory deficit in y-maze. Results are expressed as mean ± SEM (n = 8). $^{\#}P$<0.05 versus vehicle 10ml/kg normal untreated mice; ^{a}P<0.05 versus HAL-pretreated mice group. Statistical level of significance analysis by two way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Key: VINPO = Vinpocetine, HAL = Haloperidol, THP = Trihexylphenidyl HCl.

Chronic haloperidol-induced catalepsy test: Chronic administration of haloperidol caused time course and significant (p<0.001) increase in cataleptic behaviour when compared with vehicle treated control mice. However, the pretreatment of mice with vinpocetine (5, 10 and 20 mg/kg) or triheylphenidyl before haloperidol injection over a period of 21 days significantly reduced the cataleptic behaviour when compared with vehicle-haloperidol treated group (Table 3).

Biochemical evaluation

MDA Measurement: Administration of haloperidol for 2 consecutive days caused significant (p<0.001) increase lipid peroxidation (2 folds) F (6, 28) = 3.963;p<0.005 (Fig. 4a), deficits in GSH level (F(6, 28)=10.37;p<0.001) (Fig. 4b), catalase activities F (6, 28) = 6.127,p<0.001) (Fig. 4c), superoxide dismutase activities F (6, 28) = 17.11,p<0.001) (Fig. 4d) and increase in nitrite generation F (6, 28) = 5.23,p<0.01) (Fig. 4e) in the brain. Haloperidol-induced MDA and nitrite generation were attenuated by vinpocetine administration. Similarly, haloperidol induced decrease in GSH level , catalase and SOD activities were enhanced by vinpocetine administration.

Table 2:
Time course effect of vinpocetine on haloperidol-induced catalepsy in mice

Treatment	Dose (mg/kg)	15mins	30 mins	60 mins	90 mins	120 mins
vehicle	10ml/kg	1.17 ± 0.83	1.17 ± 0.28	0.54 ± 0.24	0.51 ± 0.23	0.33 ± 0.33
VINPO	20	6.33 ± 2.01	1.20 ± 0.16	0.63 ± 0.20	0.50 ± 0.22	0
HAL	1	39.17 ± 13.02$^{\#}$	30.33 ± 8.91$^{\#}$	22.67 ± 4.67$^{\#}$	74.50 ± 3.57$^{\#}$	80.83±13.81$^{\#}$
vin+HAL	5+1	8.17 ± 2.64^{d}	16.17 ± 2.38	7.50 ± 0.67	3.17 ± 0.30^{d}	4.17±0.30^{d}
vin+HAL	10+1	5.83 ± 3.54^{d}	8.16 ± 1.42^{b}	2.09 ± 0.85^{a}	2.58 ± 1.05^{d}	4.50±0.42^{d}
vin+HAL	20+1	2.00 ± 0.25^{d}	4.00 ± 0.36^{d}	2.33 ± 0.21^{b}	1.31 ± 0.15^{d}	0.49±0.13^{d}
THP+HAL	0.1+1	1.58 ± 0.07^{d}	5.5 ± 0.85^{c}	4.67 ± 0.55^{a}	4.50 ± 0.43^{d}	2.83±0.30^{d}

Values are expressed as mean±SEM (n=8). Results are expressed as mean±SEM (n = 8). $^{\#}P$<0.05 versus vehicle 10ml/kg normal untreated mice; ^{a}P<0.05; ^{b}P<0.01; ^{d}P<0.0001 versus HAL-pretreated mice group. Statistical level of significance analysis by two way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Key: VINPO = Vinpocetine, HAL = Haloperidol, THP = Trihexylphenidyl HCl.

Table 3:
Effect of vinpocetine on chronic haloperidol-induced catalepsy in mice

Treatment	Dose (mg/kg	Catalepsy scores (sec)				
		Day 5	Day 9	Day 13	Day 17	Day 21
Vehicle	10ml/kg	1.33±0.16	1.50±0.34	2.33±0.21	2.33±0.21	2.83±0.30
Vinpo	20	6.83±2.45	6.00±1.67	7.83±1.30	15.67±1.52	15.17±0.60
HAL	1	30.18±10.46$^{\#}$	29.00±5.87$^{\#}$	50.50±8.24$^{\#}$	115.00±0.77$^{\#}$	149.17±8.40$^{\#}$
Vinpo+HAL	5+1	13.32±4.87^{b}	9.17±2.96^{c}	12.35±3.51^{d}	16.17±1.90^{d}	38.00±0.73^{d}
Vinpo+HAL	10+1	5.50±1.56^{d}	7.30±1.84^{c}	7.30±0.42^{d}	10.17±0.91^{d}	12.67±0.42^{d}
Vinpo+HAL	20+1	4.85±3.24^{d}	6.75±1.89^{d}	3.50±0.42^{d}	5.16±0.54^{d}	5.83±0.79^{d}
THP+HAL	0.1+1	3.88±2.42^{d}	8.16±1.13^{c}	0.71±0.36^{d}	16.50±1.05^{d}	19.00±0.57^{d}

Results are expressed as mean±SEM (n=8). The results were analyzed by two-way ANOVA followed by Tukey post hoc tests. Significant reduction in the duration of catalepsy [$^{\#}P$<0.0001 versus vehicle-treated group; ^{b}P<0.01, ^{c}P<0.001, ^{d}P<0.0001 versus HAL (1mg/kg, i.p.) treated group}.

DISCUSSION

The present study investigated the effect of vinpocetine on haloperidol induced cognitive and motor impairments in mice. In this study, subchronic administration of haloperidol caused time course increase in catalepsy and motor deficit which is in agreement with our previous study (Adedeji *et al*., 2014). However, haloperidol-induced catalepsy in bar test, motor deficit in open field test and cognitive impairment in Y-maze task were ameliorated by vinpocetine administration. In addition, haloperidol-induced increase in oxidative and nitrosative radicals' generation as well as deficit in antioxidant enzymes activities were reversed by vinpocetine administration.

Several studies have revealed novel actions of vinpocetine, including anti-inflammation, antagonizing injury-induced vascular remodeling and high-fat-diet-induced atherosclerosis, as well as attenuation pathological cardiac remodeling (Cai *et al*., 2012; Zhang *et al*., 2018).

Cai *et al*. (2012) showed that systemic administration of vinpocetine significantly reduced neointimal formation, spontaneous remodeling, dose-dependently suppressed cell proliferation and caused G1-phase cell cycle arrest, which is associated with a decrease in cyclin D1 and an increase in p27Kip1 levels. Thus, repositioning of vinpocetine for preventing or treating neurodegenerative disorders in humans (Zhang *et al*., 2018). In this study, vinpocetine prevents catalepsy, motor-imbalance (rotarod) and cognitive deficits as well as oxidative stress induced by haloperidol.

Haloperidol produced Parkinson-like syndromes and extrapyramidal symptoms in psychiatric patients (Mohajjel-Nayebi and Sheidaei, 2010). The phenomenon of cataleptic immobility induced in mice by the use of dopamine antagonist such as haloperidol is widely used to assess nigrostriatal function in rodents (Barroca *et al*., 2019). In this study subchronic administration of haloperidol-induced cataleptic behaviour suggestive of reduced nigriostriatal signaling which was ameliorated by vinpocetine. Moreover, reduction of muscle coordination in rotarod test indicates possible neurotoxic effect of haloperidol (Kumari *et al*., 2018), which was ameliorated by vinpocetine administration. Moreover, vinpocetine prevented haloperidol-induced spatial working memory impairment in the Y-maze test.

Oxidative stress to dopaminergic neurons of substantia nigra pars compact is believed to be one of the leading causes of neurodegeneration in PD. It has been suggested that reactive oxygen species (ROS) play a role in the neuronal damage occurring in ischemic injury and neurodegenerative disorders and that their neutralization by antioxidant drugs may delay or minimize neurodegeneration (Santos *et al*., 2000). Interestingly, chronic administration of haloperidol increased MDA, nitric oxide and decreased GSH in selected brain regions (Pereira *et al*., 2003; Adedeji *et al*., 2014). Findings from this study revealed that vinpocetine mitigated haloperidol increased malondialdehyde and nitrite generation as well as reduction of GSH, catalase and SOD activities in the brain. Interestingly, in vitro studies have reported the protective effect of vinpocetine against ROS attacks (Santos *et al*., 2000; Pereira *et al*., 2003).

In conclusion, findings from this study suggests that vinpocetine prevented haloperidol-induced cognitive and motor impairments through attenuation of oxidative and nitrosative stress. Thus, vinpocetine could be a potential adjunct in the management of Parkinson disease.

Acknowledgement

Authors are grateful to Mr. M. Chijioke of the Department of Pharmacology, Therapeutics and Toxicology, College of Medicine University of Lagos for his technical assistance

REFERENCES

Adedeji HA, Ishola IO, Adeyemi OO (2014): Novel action of metformin in the prevention of haloperidol-induced catalepsy in mice: Potential in the treatment of Parkinson's disease? Prog Neuropsychopharmacol Biol Psychiatry.;48:245-251.

Ariza D, Lima MM, Moreira CG, Dombrowski PA, Avila TV, Allemand A, Mendes DA, Da Cunha C, Vital MA (2010). Intranigral LPS administration produces dopamine, glutathione but not behavioral impairment in comparison to MPTP and 6-OHDA neurotoxin models of Parkinson's disease. Neurochem Res. 35:1620–7.

Barroca NCB, Guarda MD, da Silva NT, *et al*. (2019): Influence of aversive stimulation on haloperidol-induced catalepsy in rats. Behav Pharmacol. 30(2 and 3-Spec Issue):229-238.

Beitnere U, Dzirkale Z, Isajevs S, Rumaks J, Svirskis S, Klusa V. (2014): Carnitine congener mildronate protects against stress- and haloperidol-induced impairment in memory and brain protein expression in rats. Eur J Pharmacol;745:76-83.

Björklund A, Dunnett SB. (2007): Dopamine neuron systems in the brain: an update. Trends Neurosci. 30(5):194-202.

Buege JA, Aust SD (1978): Microsomal lipid peroxidation. Methods Enzymol.;52:302-10.

Cai Y, Knight WE, Guo S, Li JD, Knight PA, Yan C. (2012): Vinpocetine suppresses pathological vascular remodeling by inhibiting vascular smooth muscle cell proliferation and migration. J Pharmacol Exp Ther. 343(2):479-488.

Duty S, Jenner P (2011): Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. Br J Pharmacol. 2011;164(4):1357-91.

Fahn S, Cohen G. (1992): The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol. 1992;32(6):804-812.

Fahn S, Przedborski S. (2008): Parkinsonism. Merritt's neurol. 10:679–93.

Frank ST, Schmidt WJ. (2003); Burst activity of spiny projection neurons in the striatum encodes superimposed muscle tetani in cataleptic rats. Exp Brain Res. 152(4):519-522.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982): Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982;126(1):131-8.

Ishola IO, Osele MO, Chijioke MC, Adeyemi OO. (2019): Isorhamnetin enhanced cortico-hippocampal learning and memory capability in mice with scopolamine-induced amnesia: Role of antioxidant defense, cholinergic and BDNF signaling. Brain Res. 2019; 1712:188-196.

Ishola IO, Akinyede AA, Adeluwa TP, Micah C (2018): Novel action of vinpocetine in the prevention of paraquat-induced parkinsonism in mice: involvement of oxidative stress and neuroinflammation. Metab Brain Dis. 2018;33(5):1493-1500.

Ishola IO, Akinyede AA, Sholarin AM. (2014): Antidepressant and anxiolytic properties of the methanolic extract of Momordica charantia Linn (Cucurbitaceae) and its mechanism of action. Drug Res (Stuttg).;64(7):368-76.

Khan MS, Tabrez S, Priyadarshini M, Priyamvada S, Khan MM. (2012): Targeting Parkinson's - tyrosine hydroxylase and oxidative stress as points of interventions. CNS Neurol Disord Drug Targets. 11(4):369-80.

Knol W, van Marum RJ, Jansen PA, Egberts TC, Schobben AF. (2012): Parkinsonism in elderly users of

haloperidol: associated with dose, plasma concentration, and duration of use. J Clin Psychopharmacol. 2012 Oct;32(5):688-93.

Kulkarni SK, Bishnoi M, Chopra K (2009):. In vivo microdialysis studies of striatal level of neurotransmitters after haloperidol and chlorpromazine administration. Indian J Exp Biol. 2009;47(2):91-7.

Kumari N, Agrawal S, Kumari R, Sharma D, Luthra PM. (2018): Neuroprotective effect of IDPU (1-(7-imino-3-propyl-2,3-dihydrothiazolo [4,5-d]pyrimidin-6(7H)-yl)urea) in 6-OHDA induced rodent model of hemiparkinson's disease. Neurosci Lett. 675:74-82.

Lotharius J, Brundin P. (2002): Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat Rev Neurosci. 3(12):932-42. doi: 10.1038/nrn983. PMID: 12461550.

Mohajjel Nayebi A A, Sheidaei H (2010): Buspirone improves haloperidol-induced Parkinson disease in mice through 5-HT(1A) recaptors. Daru. 18(1):41-5.

Nakamura T, Lipton SA. Redox modulation by S-nitrosylation contributes to protein Neurosci. 30:194–202.

Okubadejo NU, Bower JH, RoccaWA, Maraganore DM (2006): Parkinson's disease in Africa: a systematic review of epidemiologic and genetic studies. Mov. Disord.;21:2150–6.

Olanow CW (2007): The pathogenesis of cell death in Parkinson's disease –2007. Mov Disord 22(Suppl 17):S335–S342

Pereira C, Agostinho P, Moreira PI, Duarte AI, Santos MS, Oliveira CR (2003): Neuroprotection strategies: effect of vinpocetine in vitro oxidative stress models (Portuguese). Acta Med Port. 16, 401–406.

Polydoro M, Schröder N, LimaMN, Caldana F, Laranja DC, Bromberg E, Roesler R, Quevedo, J, Moreira JC, Dal-Pizzol F. (2004): Haloperidol- and clozapine-induced oxidative stress in the rat brain. Pharmacol. Biochem. Behav. 2004;78(4):751–6.

Sagara Y. (1998): Induction of reactive oxygen species in neurons by haloperidol. J. Neurochem. 71:1002–12.

Santos MS, Duarte AI, Moreira PI, Oliveira CR (2000): Synaptosomal response to oxidative stress: effect of vinpocetine. Free Radic Res. 32(1):57-66.

Sarter M, Bodewitz G, Stephens DN (1988). Attenuation of scopolamine induced impairment of spontaneous alternation behavior by antagonist but not inverse agonist and agonist β-carbolines. Psychopharmacol. 94:491–5.

Sedlak J, Lindsay RH (1968): Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. Oct 24;25(1):192-205.

Sinha AK (1972): Colorimetric assay of catalase. Anal Biochem. 47(2):389-94.

Trejo F, Nekrassov V, Sitges M (2001): Characterization of vinpocetine effects on DA and DOPAC release in striatal isolated nerve endings. Brain Res. 909, 59–67.

Tsang AH, Chung KK (2009): Oxidative and nitrosative stress in Parkinson's disease. Biochim. Biophys. Acta;1792:643–50.

Upreti GC, Wang Y, Finn A, Sharrock A, Feisst N, Davy M, Jordan RB. U-(2012): An improved Lowry protein assay, insensitive to sample color, offering reagent stability and enhanced sensitivity. Biotechniques. 2012 Mar;52(3):159-66.

Wu LR, Liu L, Xiong XY, *et al*. (2017): Vinpocetine alleviate cerebral ischemia/reperfusion injury by down-regulating TLR4/MyD88/NF-κB signalling. Oncotarget. 8(46):80315-80324.

Zhang L, Yang L. (2014): Anti-inflammatory effects of vinpocetine in atherosclerosis and ischemic stroke: a review of the literature. Molecules. 2014;20(1):335-347.

Zhang YS, Li JD, Yan C. (2018): An update on vinpocetine: New discoveries and clinical implications. Eur J Pharmacol. 819:30-34.

Niger. J. Physiol. Sci. 35 (December 2020): 209 - 212
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Histomorphometry of Rat Testes Following Intestinal Ischaemia-Reperfusion Injury and Splenectomy

Olatunji-Akioye, A.O

Department of Veterinary Surgery & Reproduction, University of Ibadan. Ibadan, Nigeria.

Summary: Intestinal Ischaemia-Reperfusion Injury as distinct from Ischaemia-Reperfusion Injury is what occurs when blood supply to the intestines is cut off either inadvertently in some gastrointestinal emergencies or during abdominal surgical interventions. The phenomenon leads to formation of reactive oxygen species and subsequent up-regulation upon reaching the spleen and consequent effects in organs of the body such as increased sperm cell abnormalities. The changes which occur in the intestine and some distant organs due to remote effects of intestinal ischaemia-reperfusion have been demonstrated but there is paucity of information on these effects on the testes with implications on fertility which was investigated in this study. Fifteen adult male Wistar rats were used for this study. Group A was the control in which a sham laparatomy was done, Group B, in which intestinal ischaemia- reperfusion was set up and C in which splenectomy was done before setting up intestinal ischaemia-reperfusion by clamping the superior mesenteric artery. The ishaemia was for an hour and reperfusion also for an hour. Following reperfusion, portions of the intestines, epididymis and testes were harvested. Histomorphometry of intestines revealed a villus height of 93.98µm, 91.44µm, and 110.48µm in control, intestinal ischaemia-reperfusion and splenectomised rats and villi width of 24.5µm, 24.6µm and 38.5µm respectively. Testicular histomorphometry revealed seminal tubular diameter as189µm, 197µm and 215µm and luminal diameter of 58µm, 59µm and 62µm. Histopathology revealed congestion, desquamation and defoliation of germinal epithelium in all three groups but control rats had sperm cells present. In IIR group tubular damage and oedema were present and sperm cells were absent but in splenectomised rat, sperm cells were present in some tubules and absent in others but no tubular damage was observed. Splenectomy does exert a protective effect on intestinal ischaemia-reperfusion by moderating the effects in remote organs, as seen in testes.

Keywords*: Intestinal Ischaemia-Reperfusion Injury, Rat Testes Histomorphometry, Splenectomy*

**Address for correspondence: oishola@cmul.edu.ng; Tel: +234-8033018908*

Manuscript received: December 2019; Revised version accepted: October, 2020

INTRODUCTION

Intestinal ischaemia-reperfusion (IIR) injury is a severe disease associated with high mortality (Nadatani *et al*., 2018). Even though it affects several organs, the intestine is the most sensitive organ to IIR injury (Sasaki and Joh, 2007). Under normal physiological conditions, the intestinal barrier protects the body from the hostile environment within the bowel lumen. However, IIR disrupts this protective function, resulting in increased intestinal permeability and bacterial translocation into the portal and systemic circulations (Kong *et al*., 1998). IIR injury may also be associated with impaired gut motility and absorption. Activation of complement and circulating leucocytes by translocated bacteria may eventually lead to the development of Systemic Inflammatory Response Syndrome (SIRS) following IIR injury (Eltzschig and Collard, 2004). A devastating consequence of IIR is the development of remote organ injury and Multiple Organ Dysfunction Syndrome (MODS) which is a leading cause of death in critically ill patients in Intensive Care Units (ICUs) with mortality directly correlating with the number of failed organ systems (Neary and Redmond, 1999). Remote organ injury following IIR has been shown to be due to neutrophil mediated reactions, and Kupffer cells of the Mononuclear Phagocyte System (MPS) play a pivotal role in this sequence of events (Tullis *et al*., 1996).

Sperm abnormalities as a result of remote effects of intestinal ischaemia-reperfusion may be an iatrogenic cause of infertility in animals (Olatunji-Akioye and Fayemi, 2014). The ability of the intestine to serve as a Reactive Oxygen Species (ROS) generator and the unique position of the gastro-intestinal system to distribute metabolites round the body lends credence to a study by Ogbuewu *et al*. (2010) who found that excessive generation of ROS by abnormal spermatozoa and contaminated leucocytes is one of the few defined aetiologies for male infertility. Male infertility is most often investigated after the female has been investigated and no abnormalities are found. Histomorphometry of testes is an indication of normalcy and changes should be consistent with changes in fertility.

This study attempts to determine the histomorphometric changes that occur with intestinal ischaemia-reperfusion injury in the testes and epididymis and how splenectomy will mitigate the effect by removal of reactive metabolites that are implicated in the pathogenesis.

MATERIALS AND METHODS

Animals and groupings: Fifteen adult male Wistar rats were divided into three groups, A, B,and C. Group A served as control, group B was the IIR group and were subjected to intestinal ischaemia by clamping the mesenteric artery

while group C were splenectomised prior to clamping the mesenteric artery to set up ischaemia.

Surgical procedure: After an overnight fast, the rats were anaesthetized using intramuscular injection of a mixture of ketamine hydrochloride (10mg/kg) and xylazine (100mg/kg). A midline laparotomy was performed after skin shaving and preparation of the abdominal wall with chlorhexidine solution. The small intestine was reflected to the left of abdominal incision and the superior mesenteric artery (SMA) was exposed. Collateral arcades from the right colic artery and the jejunal arteries proximal to the site of occlusion were ligated to avoid the variable contribution of collateral circulation to the distal ileum as described by Megison *et al*., 1990. An atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int., St. Paul, Minn., USA) was then placed across the SMA just after its origin from the aorta, avoiding occlusion of the superior mesenteric vein (SMV). Intestinal ischaemia was confirmed after fifteen minutes when the mesenteric pulsations were lost and the intestine became pale. The bowel was returned to the abdominal cavity and the incision was closed with continuous 4/0 vicryl suture. After the period of ischemia depending on group, a re-laparatomy was performed and the microvascular clamp was removed. Reperfusion was confirmed with the restoration of pulsation and colour. The bowel was left within the abdomen during ischaemia and reperfusion. Splenectomy was performed just before the occlusion of SMA in the splenectomy group.

Organ harvest included the intestine, and sections of the epididymis and testes. Samples of the small bowel tissue were fixed in10% formalin solution, embedded in paraffin wax and 5 µm sections were cut and stained with hematoxylin and eosin. Testicular tissues were fixed in aqueous Bouins' fixative for 24 hours after which they were dehydrated in graded levels of ethyl alcohol, cleared in chloroform, embedded in paraffin and sectioned in a microtome at 7µ thick. The slides were stained with haematoxylin - eosin (H and E) for histological evaluations.

Histological evaluation: Histological evaluation of the intestinal segment was undertaken by an independent pathologist who was blinded to the experimental groups from which the specimens were derived. The microscopic assessment was performed using the following grading scores: + (mild congestion and no changes in architecture), ++ (moderate congestion and minimal change in architecture) and +++ (moderate congestion and significant change in architecture) adapted from Geboes, 2003. The sections were carefully examined microscopically and the best observed areas were selected for morphometric studies. The images were captured in 20 selected areas per histological section of the small bowel mucosa with a digital camera.

A semi-quantitative grading system was applied to the testes histological variables based on the approximate percentage of the total sample involved with the individual process. Although the grading system is a reflection of the extent of the change, it generally also correlates with the severity of the change. Grade - showed nil effects seen, grade + showed moderate effects from 5-25% involvement of the overall sample, grade ++, 6-55%; grade +++, above 55% (Creasy *et al.*, 2002).

RESULTS

Intestinal morphometry: Histomorphometric analysis of intestines following IIR and Splenectomy (Table 1), revealed an average villus height of 93.98µm and 91.44µm in control and IIR rats while the villus height in splenectomised rats was significantly higher, 110.48µm. The villus width followed a similar pattern with control and IIR rats measuring 24.5µm and 24.6µm while splenectomised rats had a significantly higher measurement of 38.5µm.

Testicular morphometry: Comparing the diameter of the seminal tubule and luminal diameter, it was observed that IIR rats had the least seminal diameter of 189µm, while control rats had measurements of 197µm (Table 2) and splenectomised rats had significantly higher measurements of 215µm compared to control and IIR rats. Luminal diameter was smallest in IIR (58µm) and splenectomised (59µm) rats respectively while control rats had a significantly higher luminal diameter of 62µm.

Table 1:
Histomorphometric analysis of intestines following Intestinal ischaemia-reperfusion and splenectomy in rats

	Control	IIR	Splenectomy
Villus Height	93.98 ± 2.71	91.44 ± 4.34	110.48 ± 8.01*
Villus Width	24.5 ± 1.20	24.6 ± 2.10	38.5 ± 3.26*

Table 2:
Histomorphometry of Testes following IIR and splenectomy.

	Control	IIR	Splenectomy
Diameter of seminal tubule	198.0 ±12.06	189.0 ± 11.01	215.0 ± 10.50*
Luminal diameter	62.0 ± 2.59*	58.0 ± 5.32	59.0 ± 1.18

*Values with * are significantly different from values on that row*

Table 3:
Histopathology scores of Testes following IIR and splenectomy.

	Control	IIR	Splenectomy
Congestion	+	++	++
Desquamation and defoliation of germinal epithelium	+	++	+
Presence of sperm cells	+	-	-/+
Damaged tubules	-	+	-
Oedema	-	+	+

- nil effects seen
+moderate effects (5-25%)
++significant effects (26-55%)

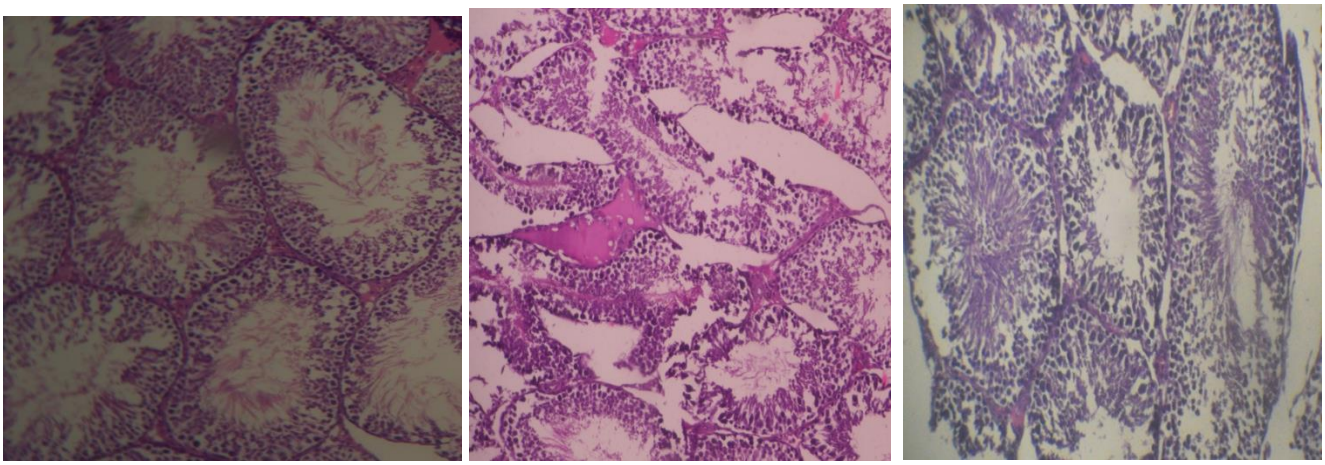


Plate 1
Testicular histology. A - Normal Testes showing discrete germinal epithelium and sperm cells within the tubules; B- Testes with damaged germinal epithelium and absence of sperm cells within the lumen; C- Splenectomised Rat testes showing germinal epithelium and sperm cells within the lumen. X100 H & E

Testicular histopathology: Histopathological evaluation of testes (Table 3) showed that control rats had congestion; desquamation and defoliation of germinal epithelium (figure 1), with sperm cells present and no damaged tubules. There was desquamation, defoliation, damaged and oedematous tubules with no sperm cells in the germinal epithelium (figure 2) in IIR rats. Tubules were damaged and oedema was also observed. In the splenectomised rat group, germinal epithelium was congested, desquamated, defoliated with scanty sperm cells (figure 3)

DISCUSSION

The histological picture of congestion and cellular infiltration in the organs should ordinarily be associated with improved oxygen delivery for the organ needs. However, the damaged nature of the blood cells makes this impossible. Although there is increased cellularity, the cells are trapped and destroyed by the phagocytic cells or they end up in the spleen. Savas *et al*. (2003) reported that experiments with leucocyte antisera proved the efficacy of splenectomy in reducing Ischaemia-Reperfusion injury by reducing the metabolites that were generated in that experiment. The splenectomised rats in this study, showed a similar histology to control animals with an amelioration of the effects of IIR. This suggests that the cells stored in the spleen probably contribute to the injury seen in IIR following reperfusion. Splenectomy removes them leading to the milder histological changes seen.

The histomorphometric changes observed suggest a response in the immediate post ischaemic period of the villus to the ischaemia and subsequent reperfusion by a width and height increase. This was probably facilitated by the chemo attraction of neutrophils which is seen to be up-regulated in IIR injury. Savas *et al*., (2003) also reported ultrastructural changes in remote organs. The changes in diameter of the seminal tubule and luminal diameter which became significantly reduced with IIR but significantly increased in splenectomised rats suggests that splenectomy may enhance the storage of sperm cells. The implications of the oedema and congestion on the gonads observed in this study suggest that IIR may be detrimental to sperm cell storage.

Shalaby and Afifi, (2008) described infertility in terms of clinical outcome of testicular torsion which establishes IR in the testicles. The mechanism of action was linked to ROS rather than oedema and congestion. However, ROS are directly responsible for the oedema and congestion that was seen in this study. The splenectomised rats were thus afforded a measure of protection as seen by the measurements as well as histology. O'Connor *et al*., 2002 detailed the effect of oestrogen on the morphology of testes and its ability to reabsorb fluid in the rete testis, efferent ducts and initial segment of the epididymis. This effect which is triggered by inflammation may be responsible for oedema seen in the IIR group of rats.

The protective effect of splenectomy in the rats lends credence to the removal of cells which would have contributed to attraction of phagocytic cells and heightened the inflammatory response. As the spleen is described as non-essential for life, it does perform storage functions and thus acts as a reservoir for blood cells in cases of urgent need. Removing the spleen in health may cause no untoward effects; it may thus be protective to splenectomise breeder animals to preclude adverse reactions in the event of an ischaemia-reperfusion injury. The importance of male fertility in breeding is such that whatever can be done to enhance it will improve the health of the entire herd.

In conclusion, splenectomy mitigates the effect of ischaemia-reperfusion injury and confers protection to the germinal epithelium and sperm cells present in the testes and epididymis

REFERENCES

Creasy, D. M., Foster, P.M.D. (2002). Male Reproductive System. In Handbook of Toxicologic Pathology (R. C. a. W. M. Haschek WM, Ed.), Academic Press, San Diego. 785-786.

Eltzschig, H. K. and Collard, C. D. (2004). Vascular ischemia and reperfusion injury. British Medical Bulletin 70: 71-86.

Geboes K. (2003). Chapter 18: Histopathology of Crohn's Disease and Ulcerative Colitis. 255-274**.**

Konig, H. E. and Liebich, H-G. (2007). Digestive System. In Veterinary Anatomy of Domestic Animals 3rd edition. Schattauer GmbH, Stuttgart, Germany. Chapter 7: 321-323.

Megison S. M., Horton J. W., Chao H., Walker P. B. (1990). A new model for intestinal ischemia in the rat. Journal of Surgical Research 49: 168-73.

Natadani Yuji, Toshio Watannabe, and Yasuhiro Fujiwara (2018). Microbiome and Intestinal Ischaemia/Reperfusion Injury. Journal of Clinical Biochemical Nutrition 63(1): 26–32.

Neary, P. and Redmond, H. P. (1999). Ischaemia–reperfusion injury and the systemic inflammatory response syndrome. In: Grace PA, Mathie RT (eds) Ischaemia–Reperfusion Injury. Oxford: Blackwell Science, 123-136.

O'Connor, J. C., Frame, S. R., and Ladics, G. S. (2002). Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. Toxicological Sciences 69, 92-108.

Olatunji-Akioye, A.O., Fayemi, O.E. (2014). Intestinal Ischaemia-Reperfusion Injury and Semen Characteristics in West African Dwarf Bucks. Nigerian Journal of Physiological Sciences Vol 29: 2, 081-083**.**

Sasaki, M. and Joh, T. (2007). Oxidative stress and ischemia-reperfusion injury in gastrointestinal tract and antioxidant, protective agents. Journal of Clinical Biochemical Nutrition 40:1–12**.**

Savas, C., Özogul, C., Karaöz, E., Delibas, N. and Özgüner, F . (2003). Splenectomy Reduces Remote Organ Damage after Intestinal Ischaemia- Reperfusion Injury. Acta chirugica belgica 103, 315-320.

Shalaby, N. M. and Afifi, O. K. (2008). Effect of Experimentally Induced Ischemic-Reperfusion on the Testis and the Possible Protective Role of L-Carnitine in the Adult Albino Rat; Histological, Histochemical and Immunohistochemical Study. Egyptian Journal of Histology Vol. 31, No 2, 233-244.

Tullis, M. J., Brown, S., and Gewertz, B. L. (1996). Hepatic influence on pulmonary neutrophil sequestration following intestinal ischemia reperfusion. Journal of Surgical Research, 66: 143-6.

Niger. J. Physiol. Sci. 35 (June 2020) 213 - 215
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Short Communication

Bleeding and Clotting Time in Different Blood Groups- A Pilot Study in a Nigerian Undergraduate Population

Ekpruke C.D.

Department of Physiology, University of Benin. Benin City, Nigeria

Summary: Many physiological factors such as ethnicity, sex and blood group have been proven to have roles to play in determining the bleeding and clotting time of an individual. Earlier studies had conflicting results about the bleeding and clotting time in an individual concerning physiological factors. The objective of the study was to study the bleeding and clotting time of an individual with respect to their sex and blood group. This cross-sectional study involved 134 undergraduate students between the ages of 17-25years. Blood group was determined using standard anti-sera, clotting time was determined using the capillary method while the bleeding time was determined using Duke's filter paper method. In this study, blood group B was predominant. The bleeding time and clotting time were significantly longer in females compared to those of males. The clotting time was longer in individuals with blood group O but the bleeding time among the blood groups was not significantly different. However, the subject size needs to be increased in further studies.

Keywords*: bleeding time; clotting time; blood group; male; female*

**Address for correspondence*: *damilola.ekpruke@uniben.edu; Tel: +2348034587346*

Manuscript received: August 2019.
Revised version accepted: July, 2020

INTRODUCTION

The time taken for a blood vessel to constrict and platelet plug formed is known as bleeding time. The time taken for the generation of thrombin which involves the actions or activations of many factors released by platelets and the damaged tissue is known as blood clotting time. These two physiological processes have been established as major ways of assessing the function of the platelet since it plays a vital role in the two processes.

There are various systems used in grouping blood. The ABO system of grouping blood remains a widely accepted system. This system was introduced by Karl in 1900 and it is based on the principle of presence or absence of antigens on red blood cell surfaces.

Many researchers over the years have reported that there is a clear relationship among blood groups, bleeding time and clotting time of an individual (Baishya *et al*., 2017; Kaur *et al*., 2015; Yasmeen *et al*., 2014). They observed that there is a lower expression of Von Willenbrand factor (vWf) in individuals with blood group O (Reddy, 2008). vWf is a glycoprotein synthesised by endothelial cells and megakaryocytes, its gene codes are located on chromosomes 12p12 and 9q34 while others found no association between bleeding time and vWf expression. Factors such as sex and race have also been implicated as having a role to play in blood clotting and bleeding time. Objectives of this study were to determine the average bleeding and clotting time in male and female young adults and also in ABO blood groups.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Physiology, School of Basic Medical Sciences, University of Benin, Nigeria. 134 apparently healthy undergraduate students during the academic year of 2017/2018 in the age group of 18-25years were selected for this study after obtaining informed consent from them. Students with any history of bleeding or clotting related disorders and on medication (NSAIDs) were excluded. This study was approved by the ethical committee of the College of Medical Sciences, University of Benin, Nigeria. Results were analyzed based on their sex and blood groups.

Blood groups of the subjects were determined using standard anti-sera. This involved the exposure of blood samples to Anti-sera A and B, thereafter observed under a microscope to determine if agglutination occurred not.

Bleeding time was determined using Duke's filter paper. This method entails the pricking of the anterior surface of the forearm with a sterile lancet and the time taken for bleeding to stop was carefully observed by using a blotting paper to blot the drop of blood on that area. Thereafter, the number of blots on that paper were counted and multiplied by 30 seconds to determine the bleeding time.

Clotting time was determined using the capillary tube method. The blood sample was taken into a capillary tube after making an incision on the skin. The clotting time was determined by breaking the capillary tube after 2minutes at a length of 1-2cm at every 30 seconds until a thread-like substance (thread-fibrin) was seen

Data analysis

Data obtained were expressed in Mean ± Standard Error of Mean (SEM) and recorded using frequency and to compare the bleeding and clotting time in the various groups. The Statistical Analysis was done using Graph pad prism 5 and P-value < than 0.05 was considered to be statistically significant

RESULTS

Data of 134 subjects were analyzed. They were aged between 17-25years. Fifty (37.31%) were males and 84 (62.69%) were females (Table 1). They were further grouped based on the different blood groups of which blood group A, B, AB and O consisted of 32 (23.88%), 58 (43.28%), 26(19.41%) and 18(13.43%) subjects respectively. It was observed in this study that blood group B was more predominant followed by A, AB and O. The bleeding and clotting times in both male and female subjects as shown in Fig. 1 showed a statistically significant difference at P< 0.05. The bleeding time in Female subjects was 4.12±0.09 mins which was longer compared to that of the male that was 3.17±0.09 mins (P=0.001).

The clotting time of female subjects was 8.15±0.12 mins compared to that of the male subject which was 7.073±0.11mins. It was significantly longer in Female subjects compared to the male subjects (P=0.001).

The blood group O, using one-way ANOVA showed a significantly longer clotting time of 8.28±0.278 mins compared to the other blood groups A (7.48±0.16 mins); B (7.53±0.15 mins); AB (7.64±0.17 mins) (P value=0.02) as shown in Fig. 2. There was no significant difference (P=0.49) in bleeding time among the different blood groups as shown in Fig. 3.

DISCUSSION

Blood group B was predominant in the selected population. The prevalence of blood group B was also reported by other scientists in consonance with our study (Patil *et al*., 2013; Roy *et al*., 2011; Abhisheth *et al*.,2011). Some of these studies carried out in Nigeria showed the contrary result, it was reported that the blood group O was prevalent (Chima *et al*., 2012). The disparity in the number of subjects that volunteered for this study may be responsible for the conflicting result.

Table 1:
Showing gender percentage among students

Sex	Frequency	Percentage
Male	50	37.31%
Female	84	62.69%
Total	134	100%

Table 2:
Distribution of Blood Groups

Blood group	Frequency	Percentage
A	32	23.88%
AB	26	19.41%
B	58	43.28%
O	18	13.43%
Total	134	100%

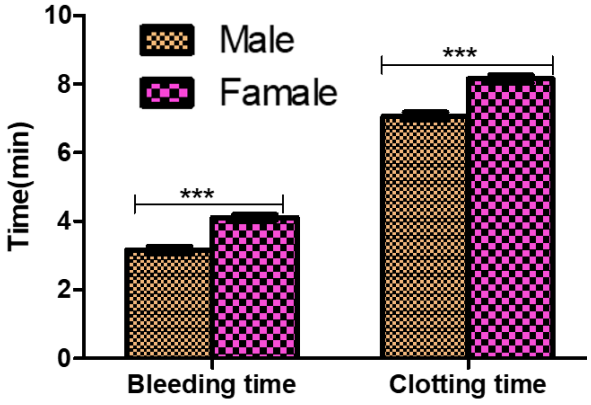


Figure 1:
A bar chart comparing the bleeding and clotting time in male and female subjects. **P < 0.05 (significant).*

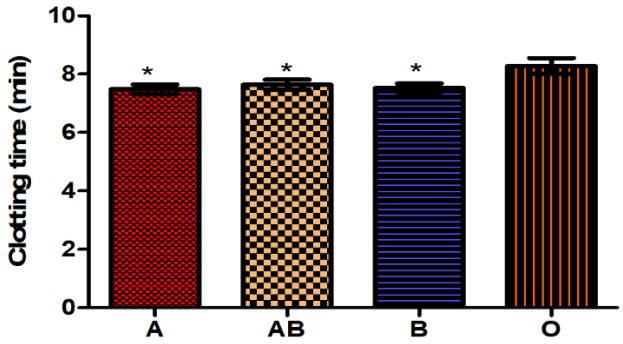


Figure 2:
A bar chart showing the clotting time in the different blood groups. *P< 0.05 (significant).

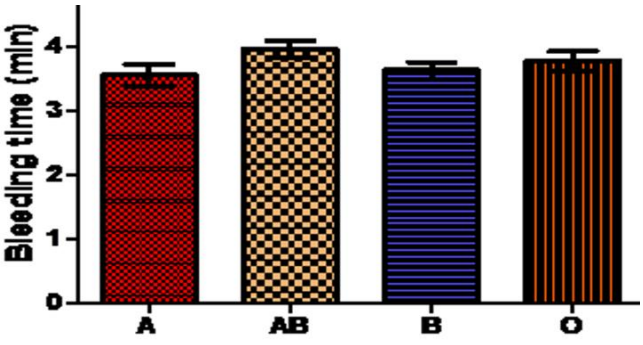


Figure 3:
A bar chart showing the bleeding time in the different blood groups.

Female subjects had longer bleeding and clotting time when compared to that of the male which was statistically significant (P value=0.001). This was in line with the report of other scientists (Baishya *et al*., 2017). Other researchers reported that there was no

relationship between the bleeding and clotting time in male and female (Mahapatra and Mishra, 2009). The presence of oestrogen in females may be responsible for the prolonged clotting and bleeding time. Oestrogen has been reported to lower the plasma level of fibrinogen thereby increasing Clotting time and dilation of blood vessels causes prolonged bleeding time (Reeta, 2017; Meena and Sunil, 2016).

Prolonged clotting time in blood group O was observed in this study and it was in line with the report of Baishya *et al*., 2017, but not in agreement with the report of other researchers which clearly stated that it was prolonged in blood group B individuals (Maharaja and Mishra, 2009) and blood group AB individuals (Yasmeen *et al*., 2014).

In the present study, a difference in bleeding time among different blood groups was not statistically significant. The prolonged clotting time in blood group O can be linked to the findings that von Willebrand factor (vWf) is higher in individuals in this group and vWf is known to play an important role in hemostasis and that its level in an individual depends on the blood group.
However, the plasma level of vWf was not estimated in this study.

In this study, blood group B was predominant while blood group O was the least dominate group among the population selected for this study. Bleeding and Clotting time were prolonged in females compared to that of the males. The clotting time in blood group O individuals was longer compared to those in the other groups while the bleeding time was not significantly different among the blood groups. However, a larger size of volunteers will be needed to confirm our findings as the number of individuals used for this study was small.

REFERENCES

Abhishekh B, Mayadevi S, Meena D, Usha KC. (2011). Distribution of ABO and Rhesus-D blood groups in and around Thiruvananthapuram. Kerala Med J.1:28–9.

Baishya R, Sarkar R, Barman B. (2017). Blood group and its relationship with bleeding time and clotting time- an observational study among the 1st MBBS students of Gauhati medical college, Guwahati. Int J Res Med Sci. 5:4147-50.

Chima OK., Mohammed TB., Aisha KG., Alhaji SA., Muhammad BM., Kwaru AH. (2012). ABO and rhesus blood groups among blood donors in Kano, North-Western Nigeria. Niger J Basic Clin Sci 2012;9:11-3

Mahapatra B. and Mishra N. (2009). Comparison of Bleeding Time and Clotting Time in Different Blood Groups. American Journal of Infectious Diseases 5 (2): 106-108.

Meena M and Sunil KJ. (2016). Distribution of blood group and its relation to bleeding time and clotting time. Int J Med Sci Public Health. 5:1-4

Patil SV, Gaikwad PB, Vaidya SR, Patil US, Kittad SD. (2013). To study the blood group distribution and its relationship with bleeding and clotting time in dental students. Asian J Medical Pharmaceutical Sci. 1(1):1–4.

Reddy VM., Daniel M., Bright E., Broad SR.and Moir AA. (2008). Is there an association between blood group O and epistaxis? J Laryngol Otol. 122(4):366–8.

Reeta B. (2017). Blood group and its relationship with bleeding time and clotting time–an observational study among the 1st MBBS students of Gauhati medical college. Int J Res Med Sci. 5:4147-50.

Roy B Banerjee, Sathian B, Mondal M, Saha CG. (2011). Blood group distribution and its relationship with bleeding time and clotting time:a medical school based observational study among Nepali. Indian and Sri Lankan students. Nepal J Epidemiol. 1(4):135–40.

Yasmeen N., Ali I. and Shaikh R. (2014). Gender-Based Blood Group Distribution and Its Relationship With Bleeding Time And Clotting Time In Medical Students. Biomed Pharm J Vol. 7(2), 619-621.

Niger. J. Physiol. Sci. 35 (December 2020): 217 – 219
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Case Report

The Foetal Anatomy and Allantoic Placenta of an African Fruit Bat (*Epomops franqueti*)

Olude M.A. [1], Aina O.A.[1], Obasa A.A.[1], Adeyanju T.E.[2], Olopade J.O.[1]

[1]*Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria*
[2]*Department of Wildlife and Ecotourism, University of Ibadan, Ibadan, Nigeria*

Summary: Bats and human biological structures are believed to be similar in terms of phylogeny, reproductive biology, and early development. Adequate knowledge of placental morphology will have important implications for research and in comparative anatomy. This report is a part of on-going studies on the African fruit bat species and is a case report from an incidental discovery of the foetus and placenta in an African fruit bat (*Epomops franqueti*) captured for research.

Keywords*: Placenta, African Fruit Bat, Foetus, Epomops*

**Address for correspondence: jkayodeolopade@yahoo.com; Tel: +234-8108587128*

Manuscript received- August 2020; Accepted- October, 2020

INTRODUCTION

Bats are often suspected culprits in zoonotic outbreaks. For instance, the *Epomops franqueti* fruit bat was one of the fruit bat species fingered previously as potential reservoirs in recent outbreak of the human filoviruses and the transmission of the Ebola and Marburg viruses (Groseth *et al* ., 2007). Several species of bats have however been reported to show wide intra-species variations in physiological baselines in response to change in geographical and environmental conditions, such as gestation lengths (Barclay *et al* .,2004; Roche *et al* ., 2020). The placental structure is one of the versatile structures capable of exhibiting extensive adaptive measures for the propagation of the bat species. Interestingly, bats and human biological structures are believed to be hosts to a wide range of similar pathogens; a relationship which may be vital for disease transmission (Annan *et al*., 2013).

Sparse studies have highlighted the allantoic placenta of several bat species but none is currently available with respect to *Epomops franqueti* (Carter and Mess, 2008; Enders *et al.*, 2009; Carter and Enders, 2016; Rodrigues *et al.*, 2019). The morphological study of the foetus and allantoic placenta will aid research understanding on the Epomops fruit bats and highlight the comparative anatomy of this species (Carters and Mess, 2008).

This histomorphological report is therefore made from an incidental collection on the foetus and placenta of the African fruit bat (*Epomops franqueti*) when in an attempt to study uterine histological architecture, one of the bats was found to be pregnant.

CASE PRESENTATION

Animal capture approval, techniques and sampling were approved by the University of Ibadan Ethical Approval Committee (UI-ACUREC/App/2016/015). Bats were obtained using mist nets in the wild and were thereafter sedated (using Ketamine (80mg/kg) and Xylazine (20mg/kg)) and transcardially perfused (with saline followed by 4% buffered paraformaldehyde) for organ histology. Incidentally, one of the bats was pregnant upon dissection. The foetal and maternal tissues were then routinely processed, using paraffin-wax embedding method, and stained with Haematoxylin and Eosin.

FINDINGS

The allantoic sac was harvested and revealed a single foetus pregnancy. Foetus appeared fully formed with limbs, external ocular and auditory features well defined. Histological features of the region reported are presented in Figure 1a-i. The foetus presented a well pronounced cervical flexure. Well-developed ventricular zones were seen at the head/brain region (1a,b), showing epithelial development that were consistent with those found lining the nasal and oral cavity (1c,e). Features that make the visceral organs readily identifiable at light microscopy but prominent forms of the tongue, heart, lungs, liver, kidney, intestines and testis were seen on section (1f, g, h). Cartilage model features of the long bones to the digits, and vertebrae with mesenchymal concretions at the periphery and some foci within the cartilaginous masses were observed (1d, f, i).

DISCUSSION

Bats usually carry a single fetus with a few are exceptions, mostly among the Vespertilionids e.g. *Pipistrellus nanus*, with two or more offspring per birth (Kurta and Kunz, 1987). From the scarce placental descriptions in bats, the African Fruit Bat (AFB) is notably absent. The features in this fruit bat are however similar to those in other Pteropodidae (Carter and Mess, 2008).

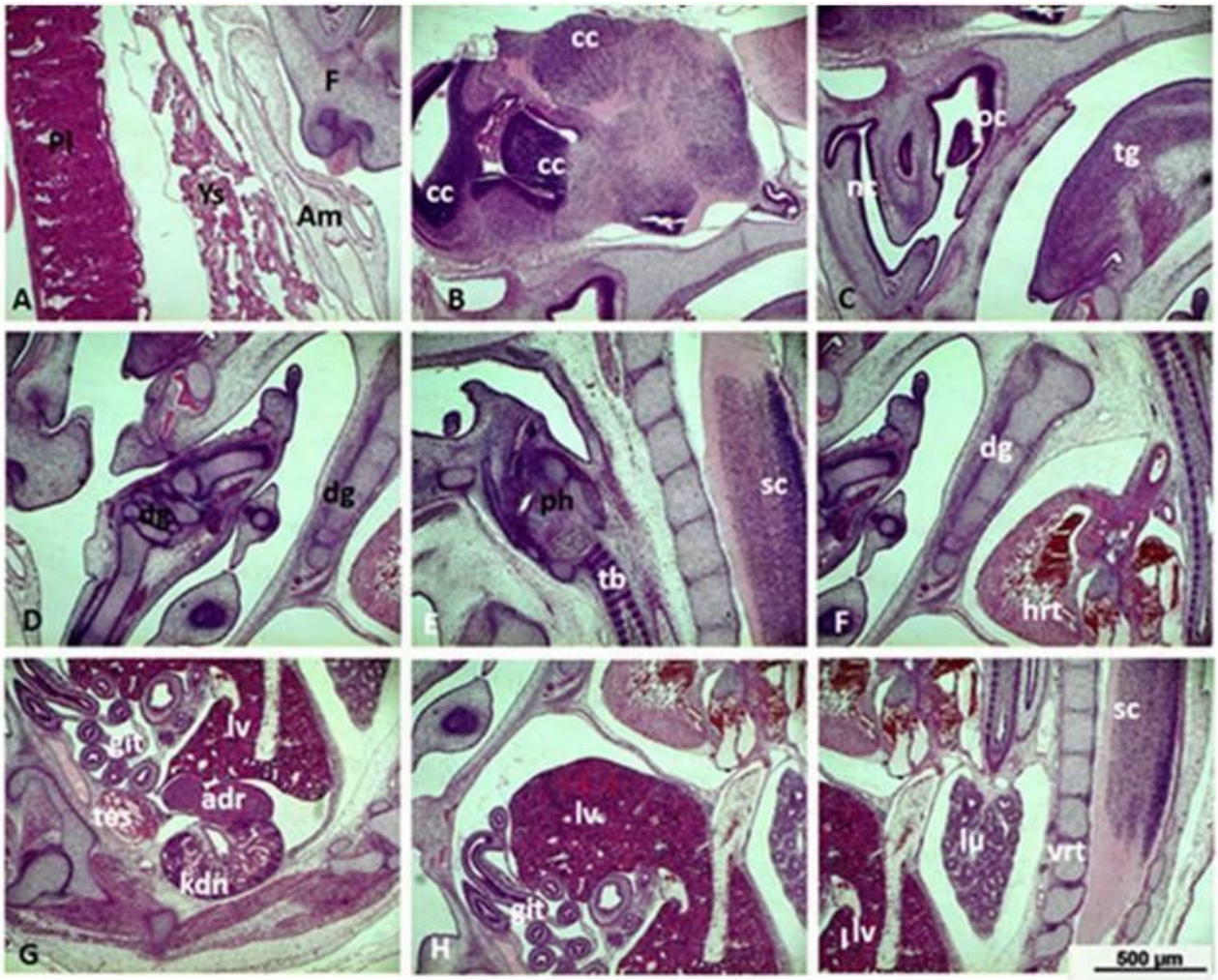


Figure 1:
Anatomical landmarks depicting structure and organ formations in the foetus of *Epomops franqueti*. (Mag x25 H&E) **A:** Showing placental and other membranes; Discoid and hemochorial placentation (Pl), Yolk sac (YS), Amnion (Am), F: Mouth part of the foetus. **B**: Showing cellular concretions at ventricular zones were seen at the head/brain region, showing epithelial development (cc) that was consistent with those found lining **C:** the nasal**. D, F** and **I:** Cartilage model features of the long bones to the digits (dg), and vertebrae with mesenchymal concretions at the periphery I: (vrt)and some foci within the cartilaginous masses were observed. (nc)and oral cavity (oc)and tongue (tg) **E:** cellular aggregations highlighting the pharynx (ph), tracheobronchi (tb) and spinal cord (sc).There were prominent forms of several viscera, **F:** heart (hrt),**G :** kidney(kdn) and testis(tes) **H:** showing liver(lv), intestinal sections (git) **I:** additionally presented liver (lv), lungs(lu) and spinal cord (sc).

The foetus can be adjudged to be in the last trimester; all the features that make the visceral organs readily identifiable at light microscopy were present, thus suggesting that organogenesis was completed. Within the limit of light microscopy, sections of the placenta can be described as chorionic villi forming interdigitations with the endometrial epithelia, consistent with epitheliochorial placentation. Endotheliochorial placentation is the ancestral condition within placental mammals where the uterine epithelium is lost but the endothelium of maternal capillaries remains intact. This is reported also in several bats e.g. the *Rhinopoma hardwickei* (Carter and Mess, 2008).

It has been suggested that the disease transmission via AFBs have been initiated by the consumption of fruits contaminated with blood and placentas during parturition of infected fruit bats. Hence, a better understanding of the placental morphology in AFBs will be useful in biomedical research for transmission and possible development of antibody sera/cultures in the AFB (Hasannin *et al* ., 2016)

In conclusion, this case report details the obvious limitation of a lone specimen and authors hereby recommend domestication, mating and gestational age studies in order to fully elucidate the AFB foetus and placental morphology.

Acknowledgement
This work was supported by Alexander von Humboldt Linkage Program grant to James O. Olopade

REFERENCES

Annan, A., Baldwin, H. J., Corman, V. M., Klose, S. M., Owusu, M., Nkrumah, E. E., and Oppong, S. (2013). Human betacoronavirus 2c EMC/2012–related viruses in bats, Ghana and Europe. Emerging infectious diseases. 19(3): 456.

Barclay, R. M., Ulmer, J., Mackenzie, C. J., Thompson, M. S., Olson, L., McCool, J., and Poll, G. (2004). Variation

in the reproductive rate of bats. Canadian Journal of Zoology. 82(5): 688-693.

Carter, A. M., and Enders, A. C. (2016). Early studies of placental ultrastructure by electron microscopy. Placenta. 41: 10-13.

Carter, A. M., and Mess, A. (2008). Evolution of the placenta and associated reproductive characters in bats. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution. 310(5): 428-449.

Enders, A. C., Jones, C. J. P., Taylor, P. J., and Carter, A. M. (2009). Placentation in the Egyptian slit-faced bat Nycteris thebaica (*Chiroptera: Nycteridae*). Placenta. 30(9): 792-799.

Groseth, A., Feldmann, H., and Strong, J. E. (2007). The ecology of ebola virus. Trends in Microbiology. 15(9): 408-416.

Hassanin, A., Nesi N., Marin J., Kadjo B., Pourrut X., Leroy É, Gembu G., Akawa P.M., Ngoagouni C., Nakouné E., Ruedi M., Tshikung D., Shongo C.P., Bonillo C. (2016). Comparative phylogeography of African fruit bats (Chiroptera, Pteropodidae) provide new insights into the outbreak of Ebola virus disease in West Africa, 2014–2016.ComptesRendusBiologies. 339, (11–12): 517-528.

Kurta A, Kunz TH. (1987). Size of bats at birth and maternal investment during pregnancy. Symp Zool Soc London. 57:79–106

Roche, N., Langton, S., Aughney, T., Lynn, D., and Marnell, F. (2020). Elucidating the consequences of a warming climate for common bat species in North-Western Europe. Acta Chiropterologica. 21(2): 359-373.

Rodrigues, A. F., Santiago, C. S., Morielle-Versute, E., Taboga, S. R., and Beguelini, M. R. (2019). Morphological variation of the female reproductive organs of the bat, Artibeus lituratus during its different reproductive phases. Journal of Morphology. 280(8): 1141-1155.