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Predictability of Metabolic Risk Factors from Hand and Body Anthropometry in Hausa Ethnic Population of Kano, Nigeria

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Summary: Validity of anthropometric tools of adiposity for metabolic risk factors (MRF) does not exhibit a universal trend, making certain adiposity tools more germane to a particular ethnic/racial group. Adiposity measures are employed to screen MRF by clinicians. The ratio of the second to fourth digit of the hand (2D:4D) has been shown to be a tight correlate of MRF. Attempts to predict MRF from hand anthropometry is relatively a new idea. The present study aims to predict MRF from digit and body anthropometry. The study recruited 266 males and 199 females of Hausa origin. Systematic random sampling was employed. Anthropometric measurements and blood pressure were obtained using standard techniques. Regression analysis was used to predict MRF, SPSS version 20 was used for statistical analyses and $P < 0.05$ was set as level of significance. MRF (serum glucose, total cholesterol, lipoprotein cholesterol, and blood pressure (BP) were predictable from 2D:4D and body anthropometric measures. Waist-to-hip ratio (WHR) was the most consistent MRF predictor. In males, WHR alone predicted TC ($R2 = 0.67$ and $P<0.0001$), HDL-C ($R2 =0.68$ and $P <0.0001$), LDL-C ($R2 = 0.67$ and $P < 0.001$) and diastolic blood pressure (DBP) [$R2 =0.43$ and $P <0.001$)]. The right 2D:4D contributed slightly to the prediction of SBP and FBG increasing the R2 value to 0.62from 0.6 for FBG and from 0.6 to 0.64 for SBP.

Keywords*: Body anthropometry, Digit anthropometry, Metabolic risk, Predictability*

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INTRODUCTION

Dislipidemia, hyperglycemia, hypertension and obesity are leading causes of morbidity and mortality globally and together constitute the metabolic syndrome (MetS) (Moller and Kaufman, 2005). The syndrome was previously thought to be a problem of developed nations, but it's prevalence in the developing nations such as Nigeria is rising alarmingly and has even been shown to assume an epidemic dimension in the last two decades (Mukadas and Misbahu, 2009; Ifeoma et al., 2011).

The use of anthropometric indices of adiposity to estimate and predict the risk of MetS by clinicians and public health physicians is a common practice globally (Tulloch-Reid et al., 2003; Ekezie et al., 2011). This practice is rooted to several documented evidences linking body adipose tissue accumulation to insulin resistance, release of pro-inflamatory cytokines such as interleukins 1 and 6 and reduction in anti-inflamatory cytokines such as adiponectin which are major steps in the pathogenesis of MetS (Kissebah et al., 1982; Lara-castro et al., 2007; Ghantous et al., 2015).

Inspite of the pathologic role of adipose tissue noted, there are convincing evidences that, in addition to the total body adipose tissue reserve, the specific anatomic site of lipid aggregation is critical in the susceptibility of an individual to MetS (Després and Lemieux, 2006; Després et al., 2008). This has recently led endocrinologist to the concept of "fat distribution rather than "fat collection" and consequently a greater attention on central and visceral adiposity (measurable by indices such as waist circumference) rather than generalized adiposity measurable by Body Mass Index (BMI) (Fontana et al., 2007; Amato and Giordano, 2014)

Although the superiority of visceral adiposity over generalized adiposity in their relationships with metabolic risk factors (MRF) has been clearly elucidated in the literature (Amato and Giordano, 2014), however ethnicity and race are documented to modulate these interrelationships such that the predictive potential of a particular adiposity measure does not demonstrate a uniform trend in all racial and ethnic populations (Tulloch-Reid et al., 2003) and may not have the same predictive ability for each MRF (hyperlipidemia, hyperglycemia and hypertension).

Currently, there are ongoing global efforts to explore other sensitive anthropometric variables to compliment adiposity tools in the risk estimation and prediction of MetS (Asuku et al., 2016; Ranvider and Manju, 2016). On this note, the ratio of the second-to-fourth ratio of the hand (2D:4D), a prenatally determined sexually dimorphic variable has recently gained attention having demonstrated strong correlation with anthropometric indices of body adiposity among Europeans (Fink et al., 2003; Fink et al., 2006), Ugandans (Abba et al., 2012) and among Nigerians (Danborno et al., 2008; Oyeyemi et al., 2016). The recent studies of Asuku et al. (2016) and that of Ranvider and Manju, 2016 revealing a strong correlation between 2D:4D and components of MetS call for a more keen attention on the probable role of 2D:4D in the prediction of MRF. The aim of the present study was therefore to pull together 2D:4D and the anthropometric indices of adiposity (BMI, NC, WC, HC, WHR, WHtR, BAI) in a linear regression model to estimate the contribution of each variable in the prediction of MRF among the Hausa ethnic group in Kano, Nigeria. This study gains its uniqueness from the paucity of data globally and among Nigerians on the role of 2D:4D in predicting MRF and scarcity of studies on the precise contribution of each anthropometric adiposity tool in predicting different MRF.

MATERIALS AND METHODS

Four hundred and sixty-five subjects who are Hausa indigenes of Kano were selected using Systematic sampling technique. Participants were recruited from outpatient units of Murtala Muhammad specialist Hospital, Khadija Memorial Hospital, the old campus of Bayero University, Kano, SU clinic Gabasawa, General Hospital Dawakin – Tofa.

The study included only subjects in the age range of 18 years to 68 years. Subjects with congenital and/or acquired digit deformity and those on medications that could interfere with any components of MetS were excluded. Ethical approval was obtained from Kano state hospitals management board and written informed consent obtained from the subjects.

Anthropometric Methods: Height was measured to the nearest 0.1cm as the vertical distance between the standing surface and the vertex of the head while the subject was standing erect in the frank forth plane and without shoes using a stadiometer. The weight was measured in kilograms using a digital weighing scale while the subject is in light clothes.

Finger Length Measurements: Digit lengths was measured on the ventral surface of the hand from the basal crease of the digit to the tip of the finger using a digital sliding caliper (MicroMak, USA) measuring to 0.01mm and reported on questionnaire. This measurement has been reported to have high degree of repeatability (Manning et al., 1998; Danborno and Danborno, 2015).

Blood pressure: A mercury sphygmomanometer was used for measuring blood pressure. Two measurements were taken, and at least 2 minutes was allowed between readings. While the diastolic reading was taken at the level when sounds disappear (Korotkoff phase V), the systolic was taken at the level when it appears. (Haffner et al., 1992).

Serum Analytical Methods

Serum glucose was measured using enzymatic method of Trinder (1969). Glucose oxidase converts glucose to gluconic acid while peroxidase converts the hydrogen peroxide to water and oxygen which also oxidizes the chromogen (4-aminophenazone) to a pink coloured complex which is measured colorimetrically at 510 nm.

$$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} \xrightarrow{\text{Hydrogen peroxidase}} H_2O + \text{Oxidised Chromogen}$$

Test tubes labeled, blank, standard and test, 1ml of glucose reagent was placed. Into the test tubes 10µl of distil water, standard solution and test serum was added to the test tubes respectively. These was then mixed and incubated at 37 ^{0}C for 10 minutes, after which the absorbance (Optical Density) of the test solution and standard was read at 505nm using the blank solution to zero the spectrophotometer

$$\text{glucose conc.} = \frac{\text{Absorbance of Test} \quad \text{x} \quad \text{Concentration of standard}}{\text{Absorbance of STD}}$$

Where the concentration of the glucose standard is 5.55 mmol/L.

Serum TC concentrations were measured using enzymatic method by Wybenga *et al.* (1970).
Total Cholesterol (TC) was measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction by-products, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a colour. Absorbance is measured at 500 nm. The colour intensity is proportional to cholesterol concentration.

$$\text{Cholesterol ester} \xrightarrow{\text{cholesterol Esterase}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterol} - 4\text{en} - 3\text{one} + H_2O_2$$

$$2H_2O_2 + 4\text{AAP} + \text{phenol} \xrightarrow{\text{Peroxides}} \text{Quinoneimine} + 4H_2O\ \text{(endpoint) RED}$$

Three test tubes labeled as test, standard and blank were earmarked and to each 1000 µl of the reagent R1 was added. 10µl sample was added to test and 10 µl standard to standard tube and 10µl distilled water to blank. The content was then mixed well and incubated at room temperature for 15mins. Reading was taken at 530 nm

$$\text{TC conc.} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \text{Conc. of standard}$$

Where concentration of the total cholesterol standard is 5.17 mmol/L.

Serum HDL-C concentrations were measured using enzymetic method of Wybenga *et al.* (1970). This method is

based on the principle that serum chylomicrons, LDL and VLDL are precipitated in the presence of phosphotungustic acid and magnesium chloride and the supernatant is treated as cholesterol. Into a clean test tube 0.5ml serum and 0.5 ml HDL reagent were taken, mixed and allowed to stand for 10 minutes. It was then centrifuged for 20 minutes at 2000rpm. Cholesterol reagent, 1ml was dispensed in to three cleaned test tubes labeled blank, standard and sample. Supernatant, 50µl was dispensed in to sample tube, 50µl of standard was dispensed into standard tube and 50µl of distilled water dispensed in to blank tube. All were mixed and incubated at room temperature for 5 min and read at 530 nm.

$$\text{Conc. of HDL} = \frac{\text{Absorbance of Test} \quad \text{x} \quad \text{Concentration of standard}}{\text{Absorbance of STD}}$$

Where concentration of the total cholesterol standard is 5.17 mmol/L

Serum LDL-Cholesterol was estimated using Friedewald equation (Friedewald *et al.*, 1972). LDL-cholesterol concentrations were calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the Friedewald's formula:
LDL-Cholesterol =TC - (HDL-C + Triglycerides/2.2) mmol/L.

Serum TG concentrations were measured using enzymetic method of Wybenga *et al.* (1970).
TG was measured enzymatically using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase and H_2O_2, one of the reaction products was then measured as described above for cholesterol. Absorbance was measured at 500 nm.

$$\text{Triglycerides} + H_2O_2 \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - PO_4 + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$H_2O_2 + 4\text{AAP} \xrightarrow{\text{POD}} \text{P} - \text{Chlorophenol Red quinoneimine}$$

Three test tubes were arranged as test, standard and blank tubes. 100 µl of triglyceride was added into each test tube and 10 µl of sample was added into sample tube while 10 µl of standard was added into standard tube. The content was mixed and incubated for 5 minutes at 37^0C. The absorbance was read at 520 nm. Serum TG concentration was calculated as follows:

$$\text{Concentration of test} = \frac{\text{Absorbance of Test} \quad \text{x} \quad \text{Concentration of standard}}{\text{Absorbance of Standard}}$$

Where concentration of the triglycerides standard is 2.28 mmol/l

Data obtained from anthropometric measurements and serum samples of participants were described using mean and standard deviation. Stepwise multiple linear regression analyses were used for prediction of MRF (BP, TC, FBS, HDL-C, LDL-C and VAI) from 2D:4D and body adiposity measures. SPSS version 20 (IBM Corporation, NY) software was used for statistical analyses and $P < 0.05$ was set as level of significance.

RESULTS

A total of 266 males (57%) and 199 females (43%) were studied. The participants had a mean age of 34.45 years and 32.06 years for males and females respectively.

Table 1:
Description of age, blood pressure, body and digit anthropometric measures of participants

	Male (n=266)		**Female (n= 199)**	
Variables	**Mean ± SD**	**Min-max**	**Mean ± SD**	**Min-max**
Age(years)	34.45 ± 13.52	18-68	32.06 ±15.18	18-65
BMI (kg/m^2)	21.98 ±3.93	14.52-34.33	22.19 ± 4.70	12.96-39.15
WC (cm)	77.28 ±11.17	57-111	76.02± 13.00	51-118.5
HC (cm)	87.01± 7.80	72.1-109.9	88.96± 9.86	65.6-136
NC (cm)	34.99± 2.29	30-42	31.58± 2.46	26.5-39.5
WHR	0.89± 0.08	0.71-1.11	0.85± 0.11	0.65-1.25
W/Ht	0.46 ± 0.06	0.34-0.65	0.48± 0.08	0.30-0.72
BAI	21.60 ± 3.71	13.88-33.90	26.61± 4.62	15.38-45.58
Height (cm)	169.15 ± 6.27	142-182.3	158.53 ± 6.83	136.9-175
Weight (Kg)	63.03 ±12.28	40.5-98.3	55.86± 12.99	36-108.9
DBP (mmHg)	82.59 ± 12.37	54-120	84.50± 12.99	60-120
SBP (mmHg)	128.07 ± 20.09	90-200	130.66 ± 21.87	95-205
RI (mm)	74.22 ± 5.45	61.17-90.46	67.97± 5.02	53.06-79.06
RII (mm)	72.56 ± 5.09	60.19-87.02	68.94± 4.48	55.42-82.09
RIII (mm)	80.12 ± 5.44	64.17-97.56	75.53± 4.98	63.13-94.26
RIV (mm)	75.63 ± 5.29	62.84-89.32	69.94± 4.51	55.41-85.35
RV (mm)	62.11 ± 5.31	47.17-85.87	57.60± 4.26	44.97-67.32
R2D:4D	0.96 ± 0.03	0.79-1.05	0.99± 0.03	0.86-1.07
LI (mm)	74.05 ± 5.36	60.33-87.47	67.77± 4.49	55.1-78.83
LII (mm)	73.32 ± 4.85	60.04-85.81	69.08± 4.40	57.19-80.44
LIII (mm)	80.50 ± 5.61	66.12-96.55	76.23± 5.56	50.09-98.92
LIV (mm)	76.03 ± 4.91	62.92-87.81	70.10 ± 4.71	57.45-82.26
LV (mm)	62.21 ± 5.09	47.46-74.36	57.69 ± 4.88	43.14-75.71
L2D:4D	0.96 ± 0.03	0.85-1.10	0.99± 0.03	0.92-1.09

DBP: diastolic blood pressure, SBP: systolic blood pressure, I: first digit, II: second digit, III: third digit, IV: fourth digit, V: fifth digit, R: right hand, L: left hand, 2D:4D: second to fourth digit ratio, BMI: body mass index, WC: waist circumference, HC: hip circumference, NC: neck circumference, WHR: waist-to-hip ratio, WHt: waist-to-height ratio, BAI: body adiposity index

Table 2
Serum indices of metabolic risk and visceral adiposity of study participants

	Male (n=120)		**Female (n= 41)**	
Variable	Mean ± SD	Min-max	Mean ± SD	Min-max
FBG (mg/dl)	84.67 ± 24.73	53.6-187.2	100.63 ± 34.90	54.6-176.4
T.C(mg/dl)	174.35 ± 32.31	123.7-256.10	187.32 ± 43.85	127.3-290.7
HDL–C(mg/dl)	44.10 ± 6.32	28-54.10	47.83 ± 6.71	38.9-60.6
TG(mg/dl)	117.18 ± 31.76	74.3-196.5	121.83 ± 29.25	80.4-165
LDL– C(mg/dl)	106.81 ± 32.44	58.14-192.82	115.12 ± 44.05	54.36-214.46
VAI	3.51 ± 1.71	1.67-9.10	4.46 ± 1.75	2.11-7.56

FBG: fasting blood glucose, T. C: total cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride, LDL-C: low density lipoprotein cholesterol, VAI: visceral adiposity index.

Table 3:
Stepwise multiple linear regression for prediction of metabolic risk factors from anthropometric measurements in males

Variables	**Model**	**R**	**R^2**	**SEE**	**F**	**P Value**
FBG (mg/dl)	1. FBG= 260.32 (W/H) + (-147.43)	0.77	0.6	15.71	176.83	<0.0001
	2. FBG= 266.07 (W/H) + 106.79(R2D:4D) + (-219.54)	0.78	0.62	15.47	93.59	<0.0001
TC (mg/dl)	1. TC= 358.48 (W/H) + (-145.26)	0.82	0.67	18.74	235.64	<0.0001
	2. TC= 358.53 (W/H) + (-38.83)	0.82	0.68	18.51	122.8	<0.0001
HDL-C (mg/dl)	1. HDL-C =70.74(W/H) + 107.17	0.82	0.68	3.6	248.5	<0.0001
TG (mg/dl)	1.TG= 340.51(W/H) + (-186.41)	0.79	0.62	19.6	194.32	<0.0001
	2.TG= 340.56(W/H) + (0.69)(Height) + (-70.41)	0.8	0.64	19.33	102.11	<0.0001
LDL-C (mg/dl)	1. LDL= 361.12(W/H) + (-215.15)	0.82	0.67	18.69	240.57	<0.0001
VAI	1.VAI= 19.56 (W/H) + (-13.90)	0.844	0.71	0.92	291.81	<0.0001
DBP (mmHg)	1. DBP=104.64 (W/H)+(-10.14)	0.66	0.43	9.34	200.78	<0.0001
SBP (mmHg)	1. SBP= 200.94(W/H) + (-50.00)	0.78	0.6	12.66	403.44	<0.0001
	2. SBP= 173.15(W/H) + 133.66(R2D:4D)+(-153.57)	0.8	0.64	12.02	238.47	<0.0001

FBG: fasting glucose, TC: total cholesterol, HDL-C: high density lipoprotein, TG: triglyceride, LDL-C: low density lipoprotein, VAI; SBP; systolic blood pressure, DBP; systolic blood pressure, W/H: waist-to-hip ratio, R2D:4D; right second to forth digit ratio, L2D:4D; left second-to-forth digit ratio, SEE: standard error of estimate.

Table 4:
Stepwise multiple linear regression for prediction of metabolic risk factors from anthropometric measurements in females

Variables	**Model**	**R**	**R^2**	**SEE**	**F**	**P Value**
FBG (mg/dl)	1. FBG= 326.05 (W/H) + (-191.68)	0.792	0.63	21.59	65.53	<0.0001
TC (mg/dl)	1. TC= 466.70 (W/H) + (-231.10)	0.9	0.81	19.18	170.09	<0.0001
	2. TC= 354.95 (W/H) + 405.41(L2D:4D) (-533.76)	0.93	0.86	16.79	117.34	<0.0001
HDL- (mg/dl)	1. HDL-C =65.30(W/H) + 106.37	0.83	0.68	3.84	82.87	<0.0001
TG (mg/dl)	1. TG= 299.08(W/H) + (-146.30)	0.87	0.75	14.79	117.41	<0.0001
LDL-C	1. LDL-C= 472.19(W/H) + (-308.21)	0.91	0.82	18.66	183.84	<0.0001
(mg/dl)	2. LDL-C= 366.03(W/H) + 385.1(L2D:4D)+ (-595.72)	0.93	0.87	16.47	124.02	<0.0001
VAI	1. VAI= 18.06 (W/H) + (-11.74)	0.875	0.77	0.86	127.37	<0.0001
DBP (mmHg)	1. DBP=81.83 (W/H)+ 14.58	0.68	0.46	9.56	168.22	<0.0001
	2. DBP=59.16 (W/H)+ 0.99(BMI) +12.14	0.74	0.55	8.72	121.38	<0.0001
	3. DBP=56.75 (W/H)+ 0.98(BMI) + 62.06(R2D:4D) + (-46.95)	0.76	0.58	8.52	88.43	<0.0001
	4. DBP=47.18 (W/H)+ 1.46(BMI) + 59.70(R2D:4D) + (-0.54)(BAI)+ (-32.66)	0.77	0.59	8.38	70.24	<0.0001
SBP (mmHg)	1. SBP= 153.98(W/H) + (-0.90)	0.76	0.58	14.29	266.53	<0.0001
	2. SBP= 141.32(W/H) + 158.99(L2D:4D)+(-146.87)	0.79	0.62	13.62	157.3	<0.0001
	3. SBP= 138.48(W/H) + 163.26(L2D:4D)+(-0.44) (Height) (-79.30)	0.8	0.63	13.32	112.89	<0.0001
	4. SBP= 121.99(W/H) + 165.36(L2D:4D)+(-0.45) (Height) + 0.71(BMI)+ (-80.43)	0.81	0.65	13.05	90.6	<0.0001
	5. SBP= 226.38(W/H) + 120.64(L2D:4D) + 7.63(BMI)+ (-2.62) (Weight) + (-7.68)(BAI) + 561.75(W/Ht) + 1.26(NC) + (51.20)	0.84	0.71	12.08	65.39	<0.0001

FBG: fasting glucose, TC: total cholesterol, HDL-C: high density lipoprotein, TG: triglyceride, LDL-C: low density lipoprotein, SBP; systolic blood pressure, DBP; systolic blood pressure, W/H: waist-to-hip ratio, R2D:4D; right second to forth digit ratio, L2D:4D; left second to forth digit ratio, SEE: standard error of estimate.

Stepwise multiple linear regressions (Tables 3 and 4) for predicting MRF shows that WHR was the strongest predictor of all the components of metabolic risks including visceral adiposity but with varying percentages of accuracy. For visceral adipose tissue estimation in the regression equation, WHR had 71% and 77% accuracy in males and females respectively and the SEE of 0.92 and 0.86 for males and females respectively (P<0.001). The regression equation showed the weakest percentage accuracy for DBP in both sexes. In males R^2 = 43%, SEE = 9.3 (P<0.001). However, in females DBP was predictable in a 2-step regression equation and the equation with the strongest prediction strength showed that DBP is predicable from both BMI and WHR with R^2 = 55%, SEE = 8.72 and P <

0.001. For the serum components, the equation with the highest predictive strength which explored only WHR in female was observed for LDL–C and TC (81% and 82% respectively) while the lowest was for FBG in males (63%). For males WHR had the highest estimation ability for HDL-C (68%) and the lowest for FBG (60%). There was however little contribution from R2D:4D in FBG estimation for males and contribution from L2D:4D in LDL-C and TC estimation in females. For BP prediction, there were also contributions from digit ratio, digit length and other anthropometric indices.

Overall VAI, BP and serum indices of metabolic risk were predictable from WHR alone or in addition to other anthropometric measurements in a linear regression model.

DISCUSSION

This observational study was conducted to investigate the predictive strength of digit ratio (2D:4D) and anthropometric measures of adiposity for visceral adiposity reserve, blood pressure and serum indices of metabolic risks among the Hausa ethnic group of Kano, Nigeria. The idea of predicting MRF from anthropometric measures of adiposity is rooted to the widely documented strong correlations between body adiposity reserves and MRF (Mathieu et al., 2009; Whitlock et al., 2009; Eckel et al., 2010; Simmons et al., 2010; Okamkpa et al., 2016). This is thought to be mediated by the role of adipose tissue in insulin resistance theory which is a major step in the pathogenesis of adverse metabolic indicators (Fujioka et al., 1987; Lara-castro et al., 2007; Ghantous et al., 2015).

However, 2D:4D on the other hand is a prenatally determined anthropometric variable whose development is essentially influenced by sex hormone (estrogen and progesterone) and genetic factors has only been shown recently to demonstrate strong and significant correlations with body adiposity measures (Asuku et al., 2018) and determinants of metabolic risk (Asuku et al., 2019; Ranvider and Manju, 2016). It is currently speculated that the genetic and hormonal determinants of 2D:4D may similarly be implicated in the development of body adiposity phenotypes. This may therefore explain why 2D:4D have demonstrated some predictive potential for MRF as observed in the current study.

The current study is therefore unique as it attempts to come up with a novel idea of pulling all the adiposity measures that have been documented to show strong relationships with MRF together with 2D:4D in a linear regression equation to predict BP, FBS, TC, LDL-C, HDL-C and VAI

The observation from the present study that WHR was the most consistent anthropometric predictor of MRF in the predictive equation may be explained from the point of view that the various anthropometric measures of adiposity shows different discriminatory powers for MRF (Pischon et al., 2008; MacKay et al., 2009). Even though wide ethnic and racial variations have been documented on the usefulness of different measures of body adiposity in estimating metabolic risks (Mbanya et al., 2015), more recent studies (Asuku et al., 2016; 2017; 2018) suggest that in most ethnic/racial groups, the anthropometric indices of central adiposity exemplified by WHR, WC and WHtR are the most implicated in the development of MRF. This is theoretically believed to be due to the close association between the amount of visceral adipose tissue reserve (a major factor in the development of insulin resistance) and values obtained from anthropometric measurements of central adiposity. This therefore implies that, WHR is the most relevant anthropometric measure of adiposity among the Hausa ethnic group of Kano which is in keeping with the findings of similar investigators amongst other racial and ethnic groups (Mbanya et al., 2015). This also agrees with the findings of Asuku et al. (2018) which revealed that WHR shows the strongest correlation with metabolic syndrome indices among the Hausa ethnic group. This finding is also strengthened by the observation in this study that WHR alone demonstrated a good predictive power for VAI which is the hallmark of metabolic syndrome phenotype,

Contrary to the findings of the current study, there are a few reports indicating that WHtR and other newer anthropometric measures of body adiposity such as BAI are more useful to some ethnic population (Bergman et al., 2011a). This suggests that ethnic specific factors significantly influences the pathophysiologic interplay between body adipose tissue reserve and MRF and also strengthens the current global recommendation (Tulloch-Reid et al., 2003) that every ethnic/racial population should strive to identify its own germane anthropometric determinants of MRF.

Interestingly, the present study identified 2D:4D as a useful anthropometric tool that contributes to the adiposity measures in MRF prediction. Since 2D:4D is established in utero by both genetic and hormonal factors and remains unchanged throughout life (Çelik et al., 2010; Umut et al., 2015), it is likely that certain phenotypes of digit ratio may have similar genetic determinants with those that predisposes to MRF. This finding on the contribution of 2D:4D to MRF prediction has added to the wide pool of body traits with which digit ratio has been documented to strongly correlate with and has identified 2D:4D as a simple, easily measurable anthropometric tool that may be useful to clinicians in predicting metabolic risks.

The variations observed in the predictive power of WHR for different measures of metabolic risk may suggest that even though WHR is superior to other adiposity tools in MRF prediction, it however demonstrates a discriminatory potential for different indices of metabolic risks. Thus the observations from this study that 2D:4D alone has strongest prediction for LDL-C in females (R^2 = 82%) and weakest prediction for DBP in males (R^2 = 47%).

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Full length Research Article

Serum Human Placental Lactogen Assays in Ultrasound Evaluated Pregnancy-Induced Hypertension; A Marker of Placental Function in Pregnancy

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Summary: Human placental lactogen (HPL) is a pregnancy-related hormone produced by the placenta. The overall functions of serum HPL impacts the developing fetus and placenta. The objective of this study was to determine the relationship between maternal serum concentration of HPL and sonographic fetal growth parameters in pregnancy induced hypertension as a marker of placental function. This prospective cross-sectional study was conducted over a 9-month period in the University of Calabar Teaching Hospital, Calabar, Nigeria that involved 100 women with pregnancy induced hypertension. An obstetric ultrasound scan was done on all the subjects and their blood was collected for HPL evaluation using Enzyme-linked Immunosorbent Assay (ELISA). ANOVA and Pearson's correlation were used to analyze the data. Maternal serum HPL had a significant positive correlation with PLA (P=0.000), EGA (P=0.000), EFW (P=0.000) and AFI (P=0.000) and a significant negative correlation with Proteinuria (P=0.047), FHR (P=0.032) and HC/AC (P=0.000). It is concluded that maternal serum HPL concentration increases as pregnancy advances and causes a significant increase in placental thickness, fetal weight and amniotic fluid volume, however, its reduction is significantly associated with the onset of pre-eclampsia, fetal distress and asymmetrical intra-uterine growth restriction. Thus, the evaluation of maternal serum HPL concentration is a reliable marker of placental function in the second half of pregnancy.

Keywords*: Human Placental Lactogen, Placenta, Fetal weight, Pregnancy induced hypertension, Pre-eclampsia, Intrauterine growth restriction*

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INTRODUCTION

Human placental lactogen (HPL) is synthesized in a progressively increasing amount by the syncytiotrophoblast and extravillous trophoblast of placenta. It is coded by the genes HPL-3 and HPL-4 within the human growth hormone variant (HGH-V) gene locus and regulates the expression of placental function (Garay *et al*., 2022; Durkovic and Mandic, 2009). Its level usually rises to between 5 and 7 micrograms at term, more than any other peptide hormone (Garay *et al*., 2022). HPL is detected as early as the 5th week of gestation in the maternal circulation and its production is not affected by stress or metabolic changes (Wilde and Oakey, 1975; Velegrakis *et al*., 2017). The serum concentration of HPL is usually low in early pregnancy but increases with the advancement of pregnancy, showing some correlation with placental weight. The overall action of the hormone, HPL, impacts on the fetus and the mother (Durkovic and Mandic, 2009; Reis *et al*., 2002).

Human placental lactogen is an important hormone of pregnancy and the most highly expressed peptide hormone of the placenta. It is actively involved in the physiological changes of the maternal metabolic process which favours a sustained increase in lipolysis that frees fatty acids for increased glucose supply to the fetus and placenta. HPL also increases maternal appetite, decreases the urge for maternal activities and drives metabolic adaptations throughout the course of pregnancy. In addition, it stimulates the production of insulin-like growth 1 (IGF-1) which is an important growth factor in the third trimester of pregnancy, stimulates DNA synthesis and acts as an insulin antagonist (Garay *et al*., 2022; Durkovic and Mandic, 2009; Bersinger and Ødegård, 2004; Yu *et al*., 2022).

Some studies have reported associations between subnormal levels of HPL and pregnancy complications such as intra-uterine growth restriction (IUGR), reduced fetal weight, threatened abortion, bleeding, placental calcification, fetal distress, intra-uterine fetal death, gestational diabetes and reduced maternal caregiving behaviour. Furthermore, low serum HPL has been reported to be associated with high level of maternal anxiety during pregnancy which could result in adverse maternal outcome such as elevated blood pressure and possibly gestational hypertension and pre-eclampsia. All these are evidence that normal levels of circulating HPL is requisite for an ideal pregnancy with a healthy mother (Garay *et al*., 2022; Durkovic and Mandic, 2009; Jeckel *et al*., 2018; Thomas, 2022).

Placental insufficiency encompasses several conditions in which the placenta does not function adequately to maintain optimal conditions for the developing fetus and this includes the secretion of subnormal concentrations of HPL into the maternal circulation (Jeckel *et al*., 2018; Manokhina *et al*., 2017). The measurement of maternal serum HPL concentration is a rapid means of determining pregnancies that might be in immediate danger and require hospital care and those with apparently healthy pregnancies (Wilde and Oakey, 1975). This is extremely beneficial to the Obstetrician as it aids the monitoring of pregnant women especially those with high-risk pregnancies (Durkovic and Mandic, 2009). Therefore, maternal serum HPL should be regarded as a valuable marker of placental function or insufficiency (Durkovic and Mandic, 2009; Reis *et al*., 2002; David and Spencer, 2022).

There is a dearth of data on studies of maternal serum levels of HPL in pregnancy induced hypertension and its effect on the growing fetus and placenta in this locality. We aimed to evaluate the relationship between maternal serum concentration of HPL and sonographic fetal growth parameters in pregnancy induced hypertension as a marker of placental function.

MATERIALS AND METHODS

Study design: This is a prospective cross-sectional study that was conducted in the Radiology Department, Obstetrics and Gynecology Department and Chemical pathology Department of the University of Calabar Teaching Hospital, Calabar, Nigeria. The duration of the study was from January 2019 to September 2019. The study population was obtained from the women who attended the antenatal clinic (ANC) of the Obstetrics and Gynecology Department of the University of Calabar Teaching Hospital, Calabar. Prior to the commencement of this study, approval was obtained from the health research ethics committee of the University of Calabar Teaching Hospital, in strict compliance with the Helsinki Declaration. The protocol number assigned to this study by the ethical committee was UCTH/HREC/33/329. Purposive sampling technique was employed for the research.

This study involved 100 subjects with singleton pregnancies between 20 and 40 weeks who had pregnancy induced hypertension (PIH). A subset of 71 subjects had gestational hypertension and another subset of 29 subjects had pre-eclampsia (proteinuria superimposed on gestational hypertension). The following were the exclusion criteria; Multiple gestation, gestational diabetes, sickle cell disease, human immune-deficiency virus (HIV), tuberculosis, congenital anomaly and chronic hypertension.

Procedure: After the routine antenatal tests and examinations were done by an Obstetrician, informed consents were obtained from the participants and they were each requested to fill the questionnaire. The subjects were made up of pregnant women with a blood pressure reading of ≥140/90 mmHg with or without a coexisting proteinuria. Proteinuria was determined by the use of urine dip sticks. The subjects with gestational hypertension (GH) were categorized into three groups.

- Mild GH = 140 – 159 mmHg for the systolic blood pressure and 90 – 99 mmHg for the diastolic blood pressure.
- Moderate GH = 160 – 179 mmHg for the systolic blood pressure and 100 – 109 mmHg for the diastolic blood pressure.
- Severe GH = ≥ 180 mmHg for the systolic blood pressure and ≥ 110 mmHg for the diastolic blood pressure.

The results obtained from the blood pressure measurements, body mass index assessment, socio-demographics and urine tests for protein of the subjects were recorded as data for this study.

Ultrasound procedure: Each subject was brought into the ultrasound suite of the Radiology Department of the University of Calabar Teaching Hospital, Calabar, by a female chaperon and appropriately positioned for the procedure. There was a head pillow on the couch to ensure comfort for the subjects. The ultrasound machine utilized for the study was an Aloka prosound SSD–3500sx (a 2-Dimensional device with Doppler facility) that has a curvilinear transducer with a frequency range of 3.5 - 5MHz (manufactured in 2008 by Aloka company limited located in Meerbusch, Germany). An ultrasonic gel was applied by the Radiologist and with a gentle motion of the transducer on the abdominal surface the fetuses were examined. The fetal anthropometric parameters were measured and these included Bi-parietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and the femur length (FL). HC/AC ratio was calculated by dividing the value of HC by the value of AC obtained from the measurements of the fetal body parts. The scanning duration for each subject was approximately 15 minutes. The entire ultrasound procedures were done by two experienced Radiologists. Estimated gestational age (EGA), estimated fetal weight (EFW), fetal heart rate (FHR) and HC/AC were recorded as data for this study. HC/AC > 1.2 signified asymmetrical intrauterine growth restriction (Peleg *et al*., 1998).

Amniotic fluid index (AFI) is derived by mentally dividing the pregnant abdomen into four quadrants by using the umbilicus as the reference point. The linea nigra divides the abdomen into left and right halves while a horizontal line that traverses the umbilicus separates the uterus into upper and lower halves. The four sonographic measurements were summed to obtain the AFI in cm. Oligohydramnios refers to an amniotic fluid index below 5cm (Efanga *et al*., 2023). The measured value of AFI for each subject was recorded as data for this study.

The plane of measurement of the placental thickness is a perpendicular line drawn from the point of insertion of the umbilical cord at the chorionic membrane of the echogenic placenta to its basal membrane. This measurement is usually done during periods of maternal relaxation and the absence of myometrial contractions (Efanga and Akintomide, 2020). The measured value of the placental thickness for each subject was recorded as data for the study.

Serum HPL evaluation procedure: The subjects were ushered into the laboratory of the Chemical Pathology Department of the University of Calabar Teaching Hospital, Calabar, for the HPL analysis. The specimen collection procedure was meticulously explained to the subjects prior to venepuncture. They were in the sitting position and well-rested for 20 minutes before blood collection from the antecubital vein was done. Four millilitres (4mls) of venous blood were collected into plain non-anticoagulated sample bottles for the HPL quantitative evaluation. The blood was allowed to clot. The clotted blood specimen was centrifuged at 3000 rpm for 5 minutes. The supernatant serum was then harvested and transferred to storage tubes and stored at -20 0C for a maximum of one week before batch analysis. Quantitative analysis was done using Enzyme-linked Immunosorbent Assay (ELISA). The microwell reader automatically plotted a standard curve of absorbance of the standards over their respective concentrations. The HPL values of the maternal serum samples were obtained from the standard curve (Human Placental Lactogen ELISA kit insert from Biovendor: Biovendor Laboratorni Medicina a.s, Karasek, Brno, Czech Republic). The maternal serum concentration of HPL for each subject was recorded as data for this study.

Statistical analysis:
The data obtained from the research was analyzed using SPSS version 20 (SPSS Inc., Chicago, IL). Appropriate descriptive and inferential statistical methods were used to analyse the data while tables and a bar chart were the means of displaying the results where applicable. One-way analysis of variance (ANOVA) test was done. Correlation was determined by using Pearson's correlation and P value < 0.05 was considered statistically significant.

RESULTS

The age of the 100 subjects involved in the study was from 16 to 39 years with a median of 32 years. The mean value of BMI was 34.03±6.56 kg/m2 which shows that the subjects were generally obese. HPL ranged from 1.80 to 8.54 microgram/ml with a mean value of 6.30±0.21 microgram/ml. The mean value of systolic blood pressure and diastolic blood pressure were 156±22.02 mmHg and 99.68±11.49 mmHg respectively. The mean value of proteinuria was 0.58±1.02. The mean EFW was 2.23±1.07 kg with a range of 0.37 to 4.77 kg while the mean PLA was 29.85±5.71 mm with a range of 15.65 to 38.60 mm. Majority of the subjects were married (67%), had secondary education (43%) and were employed (71%) (Table 1).

HPL was shown to have a significant positive correlation with PLA (P=0.000), EGA (P=0.000), EFW (P=0.000) and AFI (P=0.000) and a significant negative correlation with Proteinuria (P=0.047), FHR (P=0.032) and HC/AC (P=0.000). Both systolic (P=0.091) and diastolic (P=0.882) blood pressure were not significantly correlated with HPL (Table 2).

Table 1:
Socio-demographic characteristics of the subjects

Variables		F	Min	Max	Mean	SD
Age (years)		100	16.00	39.00	30.87	±5.02
BMI (kg/m^2)		100	18.90	63.00	34.03	±6.56
SYS (mmHg)		100	140.00	280.00	156.83	±22.02
DIA (mmHg)		100	90.00	160.00	99.68	±11.49
PROTEINURIA		100	0.00	3.00	0.58	±1.02
HPL (microgram/ml)		100	1.80	8.54	6.30	±0.21
Marital Status	**Single**	34				
	Married	67				
Educational Level	**Primary**	21				
	Secondary	43				
	Tertiary	36				
Employment Status	**Employed**	71				
	Unemployed	29				
EGA (weeks)		100	20.43	40.43	32.82	±5.40
EFW (kg)		100	0.37	4.77	2.23	±1.07
FHR (beats/min)		100	118.00	158.00	141.49	±8.69
HC/AC		100	0.90	1.30	1.04	±0.07
AFI (cm)		100	1.60	20.10	14.74	±3.33
PLA (mm)		100	15.65	38.60	29.85	±5.71

AFI – Amniotic fluid index; BMI – Body mass index; DIA – Diastolic blood pressure; EFW – Estimated fetal weight; EGA – Estimated gestational age; FHR – Fetal heart rate; HC/AC – Head circumference to abdominal circumference ratio; HPL – Human placental lactogen; PLA – Placental thickness; SYS – Systolic blood pressure. F = Frequency; Min = Minimum, Max = maximum.

Table 2:
Correlation of maternal serum HPL with the variables of the subjects (n=100)

Variables	Correlation Coefficient ®	P Value
AGE (years)	-0.140	0.165
BMI (kg/m2)	+0.062	0.539
SYS (mmHg)	-0.170	0.091
DIA (mmHg)	+0.015	0.882
PROTEINURIA	-0.200	0.046*
EGA (weeks)	+0.967	0.000*
EFW (kg)	+0.945	0.000*
FHR (beats/min)	-0.217	0.032*
HC/AC	-0.725	0.000*
AFI (cm)	+0.761	0.000*
PLA (mm)	+0.868	0.000*

(*) – P value < 0.05 is significant; AFI – Amniotic fluid index; BMI – Body mass index; DIA – Diastolic blood pressure; EFW – Estimated fetal weight; EGA – Estimated gestational age; FHR – Fetal heart rate; HC/AC – Head circumference to abdominal circumference ratio; PLA – Placental thickness; SYS – Systolic blood pressure.

Majority of the subjects were in the no proteinuria group (71%), while proteinuria +, proteinuria ++ and proteinuria +++ were made up of 10%, 9% and 10% of the subjects respectively. The mean HPL in the subjects gradually reduced from 6.48±0.24 (SEM) microgram/ml in no proteinuria to 5.54±0.64 (SEM) microgram/ml in +++ proteinuria (Figure 1). The highest mean HPL was in the

mild GIH group (6.67±1.99microgram/ml) while the least was in the moderate GH group (5.70±2.26 microgram/ml). The difference in the mean values of the three groups was not significant (P=0.110) (Table 3).

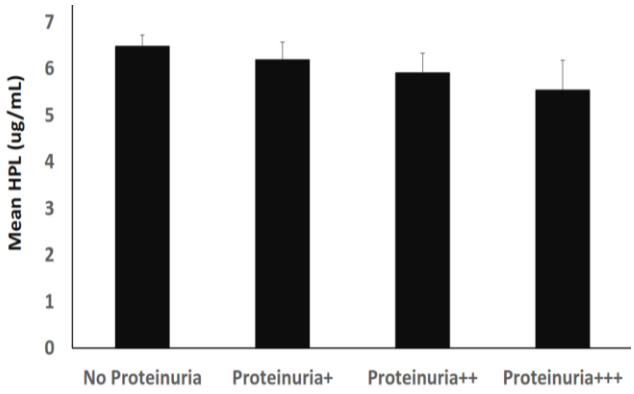

Figure 1:
Variation of mean **and standard error of mean** HPL serum concentration with the degree of proteinuria.
HPL – Human placental lactogen

Table 3:
HPL values in the GH groups

Degree of GH	n	Min µg/ml	Max µg/ml	Mean µg/ml	SD	P
Mild GH	63	1.80	8.51	6.67	±1.99	
Moderate GH	14	2.27	8.46	5.70	±2.26	
Severe GH	23	1.80	8.51	6.34	±2.08	0.110

P value – ANOVA P value; GH – Gestational hypertension; SD – Standard Deviation

DISCUSSION

The maternal serum concentration of HPL in the PIH subjects of this study was found to increase in direct proportion with the advancement of pregnancy between the 20th week to the 40th week of gestation. This association between HPL and EGA was noted to be significant (P=0.000). Our findings are in consonance with Durkovic *et al*. (2009) in Serbia, who observed that HPL increased in a linear fashion with the gestational age. McIntyre *et al*., (2000) further buttressing this fact, realized that the mean value of maternal serum concentration of HPL noted at the 28th week of gestation was increased by 69% at the 36th week of gestation. Pedersen *et al*. (1995) who evaluated HPL in maternal blood samples obtained between the 8th and 14th week of gestation found out that high levels of maternal serum HPL within this period was indicative of better fetal growth in the remainder of pregnancies. Serum HPL measurement is alleged to give information about the estimated gestational age in the first trimester of pregnancy, which could be useful in situations or locations where ultrasound services are unavailable (Sibiak *et al*., 2020).

Estimated fetal weight was observed to increase in the same manner as the maternal serum concentration of HPL did in this study. This positive correlation was found to be significant (P=0.000). In the same vein, McIntyre *et al*. (2000) reported that a significant positive correlation existed between HPL and fetal weight (P<0.001). Even in studies where maternal serum HPL were assayed at 32 weeks and 36 weeks of gestation, carried out by Higgins *et al*. (2012) and Knopp *et al*., (1985) a significant correlation between estimated fetal weight was observed with maternal serum HPL (at 32 weeks, P=0.02 and at 36 weeks, P=0.03). Still in congruence with this study, Seppala *et al*. (1970) noted that there was a significant positive correlation between maternal serum HPL concentration assayed ten days before delivery with fetal weight. In variance with our findings, Singer *et al*. (1970) found out from sampled maternal serum at birth following 50 deliveries that there was no correlation between fetal weight and HPL.

We demonstrated that HC/AC ratio had a significant negative correlation with maternal serum HPL concentration (P=0.000) which implies that an elevation of HC/AC ratio above 1.2 (that is indicative of asymmetrical IUGR) is associated with low levels of maternal serum HPL. This same trend was reflected in Bersinger *et al*.'s (2004) study that had only Scandanavian participants, who realized that maternal serum concentrations of HPL remained persistently low in IUGR fetuses as pregnancy advanced. Mittal *et al*. (2007) noted that the serum level of HPL in IUGR was lower than its value in normal pregnancy but the difference between both serum concentration was not significant (P<0.05). The evaluation of HPL can be used in the assessment of the risk for IUGR (Sibiak *et al*., 2020). However, it was reported by Kastrup *et al*. (1978) that HPL (obtained in the third trimester) was found not to correlate with fetal growth in-utero and after delivery, which differed from this study.

Spellacy *et al*. (1972) inferred after measuring the maternal serum levels of over 1000 women who had various complications of pregnancy that maternal serum HPL had no significant correlation with fetal heart pattern. However, this study revealed that maternal serum HPL was significantly correlated with FHR (P=0.032) in a negative manner such that a reduction in serum HPL likely provokes fetal tachycardia. Nevertheless, Spellacy *et al*. (1972) further stated that maternal serum concentration of HPL was of no assistance in the selection of pregnant women who needed biophysical monitoring and that it had no correlation with fetal neurological development. Thus, they insisted that there was no evidence that the use of serial HPL assays improved perinatal survival. However, Dutton *et al*. (2012) discovered that there was a significant reduction in the circulating maternal serum HPL in women whose fetuses exhibited grossly reduced movement and those who had adverse perinatal outcome, further corroborating the relevance of serial HPL assay in pregnancy.

There was no significant relationship between maternal serum concentration of HPL with the blood pressure of the subjects in this study. The highest amount of maternal serum HPL concentration in the subjects with gestational hypertension was 6.67±1.99 micrograms/ml and this was observed in the mild stage of gestational hypertension and reduced afterwards in the moderate stage of gestational hypertension. In contrast, Spellacy *et al*. (1971) observed that mean maternal serum concentration of HPL in mild gestational hypertension was lower than its value in moderate and severe gestational hypertension.

Pre-eclampsia (proteinuria superimposed on gestational hypertension) was lucidly demonstrated to exhibit a

significant negative correlation with maternal serum levels of HPL (P=0.047) in this study. It was further observed that the mean HPL in the subjects consistently decreased in value as the degree of pre-eclampsia increased. Garay *et al*. (2022) had reported that low serum HPL was associated with high level of maternal anxiety during pregnancy which could result in adverse maternal outcome such as pre-eclampsia. Similarly, it was reported by Wilde *et al*. (1975) that the mean maternal serum HPL concentration in normal pregnancies was higher than its level in pre-eclampsia. Also, Durkovic *et al*. (2009) in Serbia, equally inferred that HPL mean value in pre-eclamptics was lower (2.07±1.75mg/l) than its value in normal pregnancy (4.15±2.55mg/l). The detection of normal levels of circulating maternal HPL within the first trimester in serial assays does not necessarily portend a healthy second half of a pregnancy as was discovered by Sifakis *et al*., (2011) who reported that the subsequent development of pre-eclampsia in their subjects had been preceded by normal levels of maternal serum HPL concentrations in the first trimester.

Incongruent with our findings, Mittal *et al*. (2007) observed that maternal serum concentration of HPL was significantly lower in normal pregnancy compared to pre-eclampsia with the median value of normal pregnancy noted to be 12, 157 pg/ml while that of pre-eclamptics was 23, 076 pg/ml ($P < 0.05$), but when SGA was superimposed on pre-eclampsia, the value of serum HPL reduced to that of normal pregnancy. They postulated that the high maternal circulating levels of HPL seen in women with pre-eclampsia was possibly a compensatory mechanism geared towards preserving the fetus and a failure of this mechanism results in the development of SGA.

Appropriate development of the placenta necessary for it to meet up with the continuously increasing need of the developing fetus is achieved by an adequate circulating concentration of maternal HPL through the stimulation of IGF-1 production to induce placental cell proliferation and increase maternal blood flow to the placenta (Sibiak *et al*., 2020; Burton *et al*., 2016). Placental thickness in this study was observed to have a significant positive correlation with maternal serum HPL (P=0.000). In agreement with this study, Rasie *et al*. (2022) reported that maternal serum HPL concentration is positively related to placental thickness ($P<0.05$). In addition, Higgins *et al*., (2015) who conducted a study that had 77 subjects with normal pregnancy outcome (NPO) and 23 subjects with adverse pregnancy outcome (APO) observed that the placental length, placental weight, placental thickness and placental volume were significantly lower in APO than NPO ($P<0.0001$). Fresh placental tissues from subjects with APO had less HPL compared to its content in the placentas of those with NPO. The median value of HPL in NPO was 27.0 mg/mg while its value in APO was 10.7 mg/mg and the difference was significant (P=0.006). In an animal-based research, Jeckel *et al*. (2018) observed a reduction in the placental weight of HPL deficient pregnancies (88.1±10.31 g in HPL deficient pregnancies vs 105.5±4.42 g in pregnancies with normal HPL concentration) but the difference was not significant (P=0.110). In contrast, Higgins *et al*. (2012) inferred that there was no significant correlation between maternal serum HPL and placental weight.

Pregnant women with oligohydramnios are regarded to be at an increased risk of perinatal morbidity. In addition, women who have borderline AFI (AFI between 5 and 10 cm) have a two-fold increase in the frequency of adverse perinatal outcome. The evaluation of AFI is essential to improve the determination of high-risk pregnancies (Voxman *et al*., 2002). In this study, maternal serum concentration of HPL in the subjects was shown to have a significant positive correlation with AFI (P=0.000). Indicating that the assessment of maternal serum HPL concentration mirrors the intrauterine status of the amniotic fluid. Oligohydramnios and borderline oligohydramnios have been found to be associated with adverse effects such as IUGR, fetal distress, congenital anomalies and perinatal mortality (Madaan *et al*., 2015). Healy *et al*, (1985) in a move to illuminate the relationship between both, postulated that the human chorionic laeve in a developing pregnancy has HPL receptors on its surface that are exclusively bound to by HPL which consequently affects amniotic fluid volume and in the index study it might appear that when the maternal serum HPL concentration reduces, the binding sites for the hormone becomes incompletely occupied and this likely reduces amniotic fluid production.

The study did not take into cognizance the ethnic or regional variations that were inherent in subjects who reside in other climes but were merely traversing the city and as such the results may be heterogenous and probably applicable in other regions of the world. Future studies in this district should distinguish and relate the data obtained in the evaluation of serum HPL of pregnant subjects from diverse ethnicities or nationalities for any form of significance. A larger sample size would have been more representative of the HPL associations with fetal growth parameters, intra-uterine amniotic fluid volume and intra-uterine placenta in pregnant women within this locality. The small sample size was the limitation of this study. Future studies should employ a larger sample size which should be utilized to produce a maternal serum HPL concentration nomogram chart for pregnant women in this locality.

In conclusion, maternal serum HPL concentration increases as pregnancy advances and causes a significant increase in placental thickness, fetal weight and amniotic fluid volume, however, its reduction is significantly associated with the onset of pre-eclampsia, fetal distress and asymmetrical intra-uterine growth restriction. Thus, the evaluation of maternal serum HPL concentration is a reliable marker of placental function in the second half of pregnancy.

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Full length Research Article

Influence of pregnancy and lactation on vitamin D serum levels and antioxidant status in randomized women in Zaria

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Summary: Background: Pregnancy and lactation are normal physiological conditions that tend to influence numerous biological processes. The aim of this study was to identify the impact of pregnancy and lactation on serum vitamin D level and antioxidant status in some women in Zaria, Nigeria. Methods: A cross sectional descriptive study conducted at Ahmadu Bello University Teaching Hospital, Zaria for a period of three (3) months. Blood samples were collected, serum catalase, superoxide dismutase, lipid peroxidation and vitamin D, were determined using standard methods. Data were presented as mean ± SD, analysis was performed using one-way ANOVA and Pearson's correlation analysis. Values were considered significant at $p \leq 0.05$. Results: There was a significant difference ($p < 0.01$) serum malondialdehyde level, superoxide dismutase activity and glutathione peroxidase activity during the various trimesters of pregnancy and lactating group. However, the levels of these markers were highest in the lactating group. Furthermore, serum level of vitamin D and catalase activity was highest in the 2nd trimester and lowest in the control and lactating group respectively. Conclusion: Pregnancy and lactation altered serum level of Vitamin D, CAT, SOD, MDA and GPx suggesting a variation in oxidative stress at different trimester of pregnancy and lactation.

Keywords*: Pregnancy, lactation, oxidative stress, vitamin D*

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INTRODUCTION

There are certain physiologic conditions that alter the antioxidant status of an individual. Physiological phases such as pregnancy and lactation usually alters the level of antioxidants in women. Reports show that normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen which results in increased oxidative stress and antioxidant defences (Idonije *et al*., 2011; Ogbodo *et al*., 2014). Vitamin D has been reported to be involved in the regulation of physiologic factors that controls calcium signaling and reactive oxygen species (Berridge, 2016). The deficiency of Vitamin D causes an increase in oxidative stress (Wimalawansa, 2019). Augmented levels of oxidative stress may occur because of the increased cellular uptake and utilisation of oxygen, associated with pregnancy (Idonijie *et al*, 2011). It has been observed that pregnant women are more susceptible to oxidative damage than non-pregnant women, as evidenced by decreased antioxidants in non-pregnant women (Patil *et al*., 2007; Ugwa *et al*., 2014). Oxidative stress is the presence of reactive oxygen species (ROS) in excess of the buffering capacity of available antioxidants (Pizzino *et al*., 2017). Although the generation of ROS is a normal physiological process, their increased production in the body causes lipid peroxidation (Tiwari *et al*., 2010). The ROS scavenging mechanisms include enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH-Rx) and catalase, which limit the cellular concentration of ROS and prevent excessive oxidative damage (Scott, 1994). Furthermore, evaluating the activity of serum biomarkers of oxidative stress may provide useful information on the effects of pregnancy on antioxidant status which will further help understand the role of oxidative stress during pregnancy and lactation as such studies are lacking in the available literature. This study is therefore aimed at assessing the influence of pregnancy and lactation on serum Vitamin D level and some biomarkers of oxidative stress (malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase) in some pregnant and lactating women in Zaria.

MATERIALS AND METHODS

Demography of Study Area: Zaria is a heterogeneous city in Kaduna State. It is inhabited by about 1,018,827 people4. Kaduna is located in the North-west geopolitical zone of Nigeria, Kaduna State is one of the 36 states in Nigeria with its capital in Kaduna city. Zaria occupies a portion of the high plains of Northern Nigeria, 652.6 meters above sea level and some 950 kilometers from the coast at11o031N, 7o42E.

Study Design and Study Population: This study is a cross-sectional descriptive study that was conducted between within a period of three (3) months. A total of one hundred and seventy nine (179) women attending antenatal, family planning and immunisation clinics at the Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria, Maternal and Child Health Centre, ABUTH, Ban Zazzau, Zaria and Comprehensive Health Centre, ABUTH, Sabon Gari, Zaria were recruited for the study. A structured questionnaire about pregnancy history was administered. Blood samples were collected by a competent Laboratory Technologist at the Departments of Obstetrics and Gynaecology, ABUTH, Shika and measurements (body weight and height) were also recorded from the subjects. Body mass Index (BMI) was calculated from data collected from the anthropometric indices.

Experimental Grouping: The women were grouped as follows: (n = 179)
Group 1: Non-pregnant women: Control group (n = 15)
Group 2: Pregnant women in the first trimester (n = 40)
Group 3: Pregnant women in the second trimester (n = 41)
Group 4: Pregnant women in the third trimester (n = 53)
Group 5: Lactating women (n = 30)

Inclusion and exclusion Criteria: Healthy consenting women attending antenatal, family planning and immunization clinics at Ahmadu Bello University Teaching Hospital (ABUTH) Shika; Women and Child Centre, ABUTH, Ban Zazzau; Women Centre, ABUTH Sabon Gari, Antenatal Clinic, Sickbay, Ahmadu Bello University. Pregnant women with any disorder that affects metabolism of calcium or bone, history of endocrine, renal or liver illnesses, hypertension of pregnancy, gestational diabetes, thyroid disorders, or treatment with anti-tubercular or antiepileptic drugs in the previous 3 months were excluded from the study.

Ethical Approval Statement: Approval was obtained from the Scientific and Ethical Committee on Human Research, ABUTH, Shika, Zaria (Reference number: ABUTHZ/HREC/ K30/2014) and verbal consent was obtained from all subjects.

Collection and processing of samples: Exactly 10 ml of blood sample was collected by vein puncture, transferred to clean plain serum bottles immediately and allowed to stand at room temperature (270C) for two hours and centrifuged at 3000 g for 5 minutes to obtain the serum. The separated serum was stored at -20°C in a deep freezer at the department of Chemical pathology until needed for assay.

Determination of serum antioxidants: Serum level of vitamin D, catalase activity, superoxide dismutase activity glutathione peroxidase activity and malondialdehyde were measured using methods described by Aebi (1974), Fridovich (1989), and Ellman (1959) respectively.

Data Analysis: Data was collected, curated, cleaned up and was presented as mean ± SD. Analysis of data was done using One Way ANOVA followed by Holm-Sidak post hoc test and level of significance was tested at $P<0.05$. Pearson's correlation analysis was done to determine the inter-relationship between the variables.

RESULTS

Figures 1, 2 and 3 show the pattern of changes in concentration of vitamin D, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in the serum of subjects.

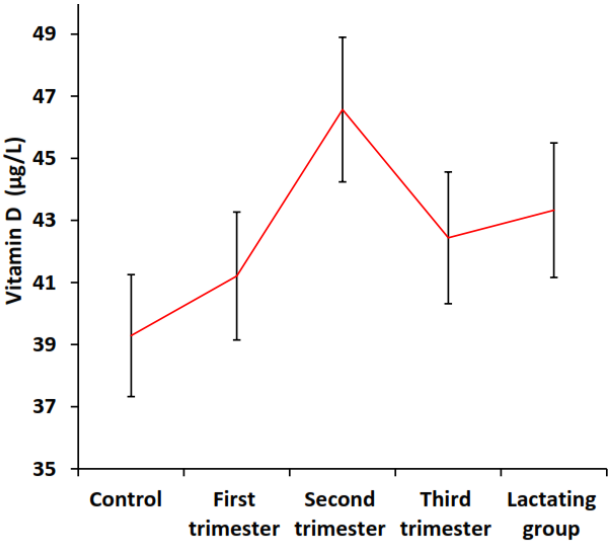

Figure 1:
Changes in the serum concentration of vitamin D in control, Pregnant and lactating women in Zaria, Nigeria

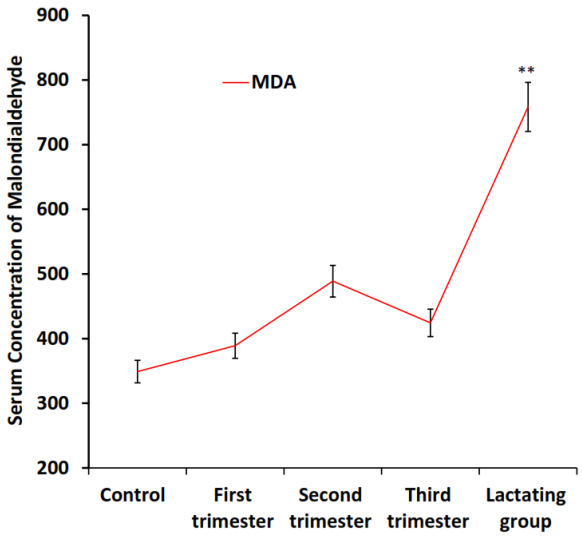

Figure 2:
Pattern of change in serum concentration of malondialdehyde in control, pregnant and lactating women in Zaria, Nigeria

The Vitamin D concentration was highest in the 2nd trimester group (46.50 ± 20.90) and lowest in the control group (39.29±4.51), MDA activity was highest in the lactating group (349.03±102.25 nmol/mg protein) and this difference was statistically significant ($p < 0.01$). There was a statistically significant ($P < 0.001$) increase observed in the activity of SOD between the different groups. This difference was observed in the lactating group, where it was higher, 14.87±1.61 U/ml. The GPx activity was also highest in the lactating group 47.43±26.00 ug/ml; and lowest in the control (11.71±1.83 ug/ml), and this difference was statistically significant ($p < 0.05$). The activity of serum CAT was highest (17.56±8.81 u/mg) in the 2nd trimester and lowest in the lactating group (3.14±0.78 u/mg), However, the difference was not statistically significant ($p > 0.05$).

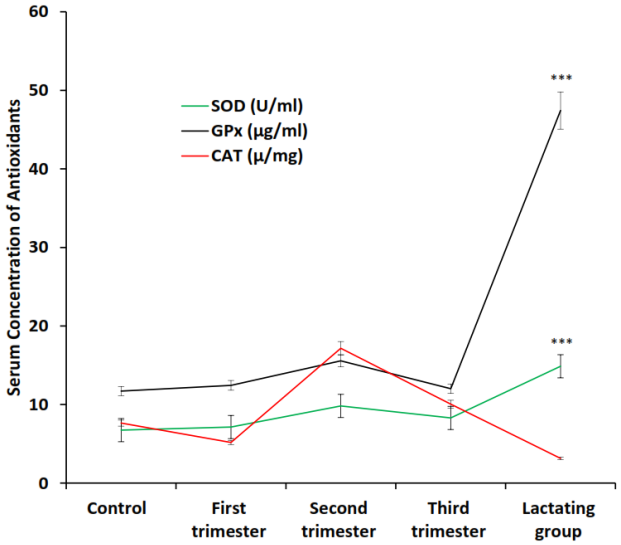

Figure 3:
Pattern of change in serum concentration of superoxide dismutase, glutathione peroxidase and catalase in control, pregnant and lactating women in Zaria, Nigeria.

DISCUSSION

Pregnant women are at an increased risk of elevated inflammation and oxidative stress especially during the third trimester. These conditions might result from micronutrient deficiency, increased maternal adipose tissue, and production of hormones by the placenta (Asemi *et al*., 2016).

It can be inferred that pregnant women with increased serum levels of inflammatory biomarkers are at elevated risk of adverse pregnancy outcome, premature delivery, and disturbances of calcium homeostasis. To reduce maternal and foetal complications resulting from unfavourable metabolic profile, various strategies have been proposed such as the consumption of antioxidants, calcium and vitamin D supplementation, which help to reduce systemic inflammation and oxidative stress (Asemi *et al*., 2016).

The result from this study showed that serum malondialdehyde concentration was highest in the 2nd trimester of pregnancy and lowest in the lactating group. Higher MDA levels in the 2nd trimester than non-pregnant control has been reported in pregnancy due to increased generation of oxygen radicals following increased oxygen demand in pregnancy and the reduction in the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Ugwa *et al*., 2014)

In a study by Fedirko *et al*., 2010, it was observed that antioxidant enzymes in humans function in combination with low-weight antioxidant compounds. Vitamin D activates the expression of antioxidant enzymes, which may not function properly in the antioxidant-depleted environment. Therefore, it is possible that vitamin D effects on the oxidative DNA are modified by the presence or absence of various pro-oxidant or antioxidant exposure. Thus, the use of Vitamin D3 supplements may decrease oxidative DNA damage in mucosa cells.

This study is in agreement with this report in that there was an observed correlation between Vitamin D with SOD and GSH which are antioxidant enzymes. Similarly, in an earlier study by Ekici *et al*, (2009), increased GSH activity was seen with Vitamin D3 supplementation in both cortex and corpus striatum in rats. Zhang *et al*., 2014 demonstrated that vitamin D supplementation in pregnant women resulted in increased GSH levels and a significant difference in plasma MDA levels. Asemi *et al*. (2014) had previously presented data in support of this, revealing that vitamin D supplementation significantly increased GSH levels just as There was strong negative correlation between vitamin D and calcium as well as vitamin D and phosphate (Avidime *et al*. 2022).

In the light of the findings in this study, it seems reasonable to speculate that the synthesis of CAT may reduce oxidative stress in the uterine environment especially during the second trimester. In addition to the suggestion that CAT might come from different sources in the serum. The pregnant human myometrium might be very efficient in the elimination of MDA (Biberoglu *et al*., 2016). Thus, the higher concentration of MDA, SOD, GSH and also CAT, in the serum samples during the 2nd trimester and indeed significantly in the lactating group (SOD), may reflect the overflow of these markers from the uterus to the circulation. It could also be that there is higher placental oxygen in the 2nd trimester than in the 1st trimester. This may possibly explain why MDA and CAT levels were observed to be low during the first trimester. A similar relationship has been previously demonstrated between the placenta and umbilical cord blood in pregnant women (Wang *et al*., 1996).

In accordance with the ischemia-reperfusion phenomenon, re-oxygenation would facilitate the transfer of oxidation markers from the myometrium to the maternal circulation, not only to play a local protective role, but also as a means of responding to systemic oxidative stress (Biberoglu *et al*., 2016). An alternative explanation could be that, independent from the systemic antioxidant process, a well-functioning myometrial system might be active enough to rescue the pregnant myometrium from reactive oxygen species, as reflected by the values obtained in the 2nd trimester when compared with the control and the 1st trimester values obtained in this study. Additionally, the reactive oxygen species may promote local myometrial increase in oxidative stress, which are rapidly detoxified, thereby elevating oxidative markers in the circulation only slightly and for a short time, which also explains the high

MDA and antioxidant levels in the circulation by the 2nd trimester observed in this study (Biberoglu *et al*., 2016).

It may well be that the increased levels of antioxidants enzymes in serum samples of pregnant women observed in this study is an indication of the effort to compensate for the elevated MDA concentration in serum, especially during the 2nd trimester. Another finding of this study is that serum CAT activity was reduced though not statistically significant during lactation. This may be attributed to the fact that the subjects were drawn from a population of women lactating at different stages (i. e. colostrum, transitional and mature milk). Another way to put the contradictory findings into perspective is the methodological problems in measuring free radicals and absolute levels of in vivo oxidative stress. In the present study, we measured four basic oxidative stress and antioxidant activity markers. Given that not all markers change in the same direction, measuring more markers makes the interpretation of the results more problematic. Moreover, to what extent the tissue-specific oxidative damage is reflected systemically and is easily measured in the serum sample is not too clear (Biberoglu *et al*., 2016).

In conclusion, we believe that increased generation of oxygen free radicals (oxidative stress), defense mechanisms and calcium homeostasis are closely associated. Yet, the oxidative stress in circulation must be interpreted with great caution. The increased or decreased oxidative stress marker levels in the serum samples may represent reactive changes in the context of an endothelial or inflammatory status. Thus, larger multicenter studies may be required to clarify the discrepancies seen.

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Full length Research Article

Calculated Nutritional Indices in Symptomatic Hospitalized Nigerian Covid-19 Patients

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Summary: Multifaceted approaches are needed to control the ongoing COVID-19 pandemic, therefore assessing the patients' nutritional status is desirable to justify the suggestion of biochemical nutritional markers or nutritional indices in the prognosis of COVID-19. This longitudinal study determined biochemical nutritional markers (albumin, prealbumin and total cholesterol) and nutritional indices [Controlling Nutritional Status (CONUT) score and Prognostic Nutritional Index (PNI)] in symptomatic hospitalized COVID-19 patients compared with control. These parameters were related to age, sex and days of admission of the patients. Plasma obtained were analyzed for biochemical nutritional markers and indices calculated. Data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., USA) version 20.0. The mean prealbumin (PAB) and total cholesterol (TC) levels were significantly lower in COVID-19 patients compared to control ($P<0.05$). PNI classified 90% of COVID-19 patients as well-nourished while CONUT score classified 75.6% of COVID-19 patients as mildly malnourished. In COVID-19 patients at discharge, the mean level of TC was significantly increased compared with COVID-19 patients at admission. The mean albumin level in patients with ≤10days of admission was significantly lower when compared to those with those having >10days of admission. There were no significant differences in the PNI and CONUT scores of the participants in relation to age, gender and days of admission. This study concluded that Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-COV 2) infection affects certain biochemical nutritional biomarkers and that PNI and CONUT could be use as cheap, reliable and affordable nutritional prognostic tools in the management of COVID-19 patients.

Keywords*: SARS-CoV-2 infection; Nutritional indices; Gender; Length of admission*.

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INTRODUCTION

COVID-19 is an infectious viral disease caused by coronavirus SARS-CoV-2. The most common symptoms in hospitalized COVID-19 patients are fever (up to 90% of patients), dry cough (60%-86%), shortness of breath (53%-80%), fatigue (38%), nausea/vomiting or diarrhoea (15%-39%), and myalgia (15%-44%) (Docherty *et al*., 2020). The symptoms appear after an incubation period of approximately 5.2 days (Li *et al*., 2020) and about 97.5% of people develops symptoms within 11.5 days of infection (Lauer *et al*., 2019).The virus is transmitted through respiratory droplets, contact and fomites with high degree of infectivity(Perlman, 2020). SARS-CoV-2 has two principal strains: 'L type' (70%) and 'S' (30%), with the L type being more aggressive and contagious than the S type (Guo *et al*., 2020; Tang *etal*., 2020). Current SARS-CoV-2 pandemic has brought tremendous pressure on public health and medical systems worldwide which requires concerted effort (Timor-Lester, 2020). It has been observed that people that are immune-compromised are highly prone to SARS-CoV-2 infection and least likely to recover (Long *et al*., 2020). Also, older adults and people of any age with serious underlying medical conditions might be at higher risk for severe forms of COVID-19 disease (Long *et al*., 2020). Therefore, susceptibility to SARS-CoV-2 may be related to nutritional status among other factors.

Nutrition has attracted more attention in various clinical fields. Generally, malnutrition is considered an indicator related to increased morbidity and mortality (Vandewoude*et al*., 2019). Therefore, the assessments of early nutritional status of different diseases are important means of identifying malnutrition, nutritional risks, and possible benefit of nutritional interventions. Traditional nutritional measurements (height or Body Mass Index) or laboratory indicators (albumin, prealbumin and total cholesterol levels) were used to assess the nutritional status (Cook *et al*., 2005) and these assist in the prognosis of COVID-19 patients. An expert consensus on COVID-19 suggested that nutritional risk screening should be conducted among in-hospital COVID-19 patients (Barrazzoni *et al*., 2020).This is supported by evidence that nutritional status on admission is closely associated with the prolonged hospitalization or increased mortality (Arodiwe *et al*., 2015; Das, 2015; Anaszewicz and Budznski, 2017). Mearrs, (1996) reported that early recognition of protein malnutrition shortens length of hospital stay and improve patient outcomes.

It has been shown that malnutrition is linked with inflammatory responses and the development of autoimmune diseases (Fukuda *et al*., 2005; Harrison *et al*., 2012). A normal nutritional state is the prerequisite for regulating oxidative stress and inflammatory process which ultimately have impact on immune system (Wu, 2020). Nutrition deficiency leads to different complications and act as negative prognostic factor in COVID-19 patients. Host nutritional status can predict the susceptibility of individuals to be infected with SARS-CoV-2. Malnutrition may promote viral replication by altering both the innate and adaptive immune responses leading to increase susceptibility to infections (Najera et al., 2004).

Controlling Nutritional Status (CONUT) score and Prognostic Nutritional Index (PNI) are increasingly being used as markers of patients' nutritional status (Narumi *et al*., 2013; Zhou *et al*., 2019). CONUT was used to assess early detection of patient poor nutritional status (Ignacio *et al*., 2005). It is calculated from the combination of serum albumin concentration, total blood cholesterol levels, and total peripheral lymphocyte count. Moreover, Prognostic Nutritional Index (PNI) used as a nutritional and inflammation-based index was derived from the serum combination of serum albumin level and total lymphocyte count. Albumin or total lymphocyte count has been used as marker of nutritional status (Gonzalez *et al*., 2011; Bharadwaj *et al*., 2016). Since components of CONUT and PNI changes in COVID-19 patients, it is expected that the CONUT and PNI will be useful in the prognosis of COVID-19 disease.

MATERIALS AND METHODS

Study design and subject's population: This study involved a total of ninety-five hospitalized COVID-19 patients aged between 15-80years (28 females and 67 males). They were confirmed to be infected with SARS-CoV-2 using nucleic acid Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) on nasal and pharyngeal swab specimens according to WHO guideline (Li, 2020). Excluded among COVID-19 patients were those with co-morbidities and who didn't consent for follow-up study. The control subjects consist of 45 uninfected healthy adults (25 males and 20 females) aged between 18years and 65years.

Sample collection: A total of 5ml venous whole blood was collected. Three milliliters (3ml) venous blood sample was collected using pyrogen-free needle and syringes from each participant and dispensed into lithium heparin bottle for albumin, prealbumin and total cholesterol estimation. The remaining 2ml was dispensed into EDTA bottle for total lymphocyte counts (TLC). The blood samples of the patients were collected on the day of admission and another blood sample were collected at the point of discharge when patient had been tested negative for SARS-CoV-2 virus using nucleic acid Reverse-Transcriptase Polymerase Chain Reaction.

Laboratory Analysis

Biochemical nutritional parameters: Plasma albumin and prealbumin were determined using Enzyme Linked Immunosorbent assay kit (Immunology Consultant Laboratory Incorporated) and total cholesterol level was determined using oxidase-peroxidase method (Randox kit) as described by the manufacturer.

Principle of the Enzyme Linked Immunosorbents Assays (ELISA): Human albumin and prealbumin ELISA kits were obtained from Immunology Consultant Laboratory Incorporated, Portland, United State of America with batch number E-80AL and E-80PRE respectively. The procedures of the ELISA kits are based on the Sandwich-ELISA principle as described by the manufacturer. In this assay the prealbumin or albumin present in samples reacts with the anti-prealbumin or anti-albumin antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-prealbumin or albumin antibodies conjugated with horseradish peroxidase (HRP) were added. These enzyme-labeled antibodies form complexes with the previously bound prealbumin or albumin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is directly proportional to the concentration of prealbumin or albumin in the sample tested. Thus, the absorbanceat 450 nm is a measure of the concentration of prealbumin or albumin in the test sample. The quantity of prealbumin or albumin in the test sample can be interpolated from the standard curve constructed from the standards.

Cholesterol estimation (Oxidase-peroxidase method): Kit was obtained from Randox Laboratories Limited, County Antrim, BT29 4QY, United Kingdom. Plasma Cholesterol was estimated using the enzymatic assay method (Allain *et al*., 1974)**.** Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from Hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. A coloured complex that can be measured spectrophotometrically is formed. The intensity of the colour indicates the concentration of cholesterol in the sample (Allain *et al*., 1974).

Total Lymphocyte Counts: This was done using The Sysmex auto-analyser. The Sysmex XN-450 is a multi-parameter quantitative automated hematology analyzer whose function is based on the hydrodynamically focused impedance measurement, the flow cytometry method (using a semiconductor laser) and the SLS-hemoglobin method.

Nutritional Indices

Controlling Nutritional Status score (CONUT Score): The CONUT score was calculated using serum albumin concentration, total cholesterol concentration and total peripheral lymphocytes cell count (Ignacio *et al*., 2005).CONUT scores were used for classification as: Normal scores (0-1), Mild scores (2-4), Moderate scores (5-8) and Severe scores (≥9) (Ignacio *et al*., 2005)

Prognostic Nutritional Index (PNI): PNI was calculated as 10 x Serum Albumin value (g/dL) + 0.005 x Total

peripheral blood lymphocyte count (unit/L) (Onodera *et al*., 2005). PNI scores were used for classification as follows: Severe score = PNI < 35, Moderate score = 35≤PNI<38 and Normal score = PNI≥38 (Onodera *et al*.,1985).

Statistical analysis: Data obtained were analysed using Statistical Package for Social Science (SPSS) (version 20). Genders, age, days of admission were presented as frequencies and percentages in each category. Mean between two groups were compared using Student's t-test. Pearson's correlation was used to test the relationship between variables. Difference was considered significant when the p - value was less than 0.05.

Ethical Consideration: The study was conducted after approval was obtained from 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.

RESULTS

Most COVID-19 patients (89.5%) were well-nourished based on Prognostic Nutritional Index and 75% were mildly-malnourished based on Controlling Nutritional Status Score. High percentage (60%) of the patients spent ≤ 10 days on admission (Table 1).

Table 1:
Days of admission and classification of COVID-19 patients into severity groups based on CONUT and PNI.

Variable	Categories	Frequency	Percentage (%)
DOA	≤10 days	57	60
	>10 days	38	40
CONUT	Normal	24	25
	Mild	71	75
	Moderate	0	0
	Severe	0	0
PNI	Normal	85	89.5
	Moderate	10	10.5
	Severe	0	0

***Abbreviations**: DOA = Days of Admission, CONUT = Controlling Nutritional Status Score, PNI = Prognostic Nutritional Index*

Table 2:
Comparison of age and biochemical nutritional markers in COVID-19 patients with un-infected controls.

Variables	COVID-19 Positive (n=70)	COVID-19 Negative (n=45)	t-values	p-values
Age (years)	33.80 ±11.80	37.11 ±9.06	-1.111	0.270
Albumin (ng/ml)	4.99 ± 0.70	4.68 ±0.67	1.729	0.088
Prealbumin (ng/ml)	33.82 ±4.53	36.50 ±3.08	-2.412	0.018*
TC (mg/dL)	121.43 ±35.98	163.33 ±27.05	-7.389	0.000*

TC = Total cholesterol
**Significant at p<0.05*

Biochemical nutritional markers of COVID-19 patients were compared with un-infected control. The means levels of prealbumin (p = 0.018) and total cholesterol (p = 0.000) were significantly lower in COVID-19 patients compared with control (Table 2). The mean level of total cholesterol was significantly increased in COVID-19 patients at discharge compared with at admission (p = 0.018) (Table 3).

Table 3:
Comparison of biochemical nutritional markers in COVID-19 patients on admission with those at discharge.

Variables	On admission (n=25)	At discharge (n=25)	t – values	p– values
Albumin (ng/ml)	5.32 ± 0.11	5.27 ±0.0.17	1.357	0.205
Prealbumin (ng/ml)	24.33 ±9.48	26.32 ±10.52	-0.780	0.453
TC (mg/dL)	110.55 ±19.45	149.91 ±55.99	-2.818	0.018*

*TC = Total Cholesterol, *Significant at p<0.05*

Table 4:
Comparison of biochemical nutritional markers in male with female COVID-19 patients.

Variables	Males (n= 49)	Females (n= 21)	t – values	p – values
Albumin (ng/ml)	5.06 ±0.67	4.98 ±0.83	0.374	0.710
Prealbumin (ng/ml)	33.99 ±3.58	32.85 ±6.62	0.829	0.411
TC (mg/dL)	124.84 ±37.42	125.50 ±39.69	-0.058	0.954

*TC = Total Cholesterol, *Significant at p<0.05*

Table 5:
Comparison of biochemical nutritional markers of COVID-19 patients aged less than 40 with COVID-19 patients aged greater than or equal to 40 years.

Variables	Age <40years (n = 51)	Age ≥40 years (n = 19)	t – values	p – values
Albumin (ng/ml)	5.07 ±0.75	4.95 ±0.62	0.542	0.590
Prealbumin (ng/ml)	33.79 ±5.05	33.30 ±3.42	0.350	0.728
TC (mg/dL)	119.65 ±29.78	141.62 ±53.80	-1.866	0.068

*TC = Total Cholesterol; *Significant at p<0.05*

Table 4 shows biochemical nutritional markers of male COVID-19 patients compared with female COVID-19 patients. All the variables showed no significant differences between both genders. Table 5 shows biochemical nutritional markers of COVID-19 patients aged<40 and ≥40 years. All the variables show no significant differences between the two groups. Table 6 shows comparison of biochemical nutritional markers of COVID-19 patients based on their days of admission. The mean albumin level of COVID-19 patients with ≤10days of admission was significantly lower when compared withCOVID-19 patients having >10days of admission (p = 0.030). Using CONUT score, more (39.3%) female COVID-19 patients than male (35.8%) patients, more patients (38.5%) ≥40 years of age than < 40 years of age (27.5%) and more patients (30%) with ≤10days hospital stays than >10days (29%) hospital stay

were classified as having normal nutritional status (Table 7).Using PNI, more (82.1%) female COVID-19 patients than male (80.6%) patients, more patients (80.8%) ≥40 years of age than < 40 years of age (76.8%) and more patients (84.2%) with >10days hospital stays than ≤10days (77.2%) hospital stay were classified as having normal nutritional status (Table 8). Pearson's correlation coefficients between age, days of admission with nutritional markers or indices in COVID-19 patients showed no significant correlations (Table 9).

Table 6:
Comparison of biochemical nutritional markers ofCOVID-19patients based on days of admission.

Variables	DOA ≤10 days (n = 42)	DOA >10 days (n = 28)	t – values	p – values
Albumin (ng/ml)	4.86±0.67	5.28±0.71	-2.236	0.030*
Prealbumin (ng/ml)	33.85±4.21	33.39±5.26	0.363	0.718
TC (mg/dL)	123.52±40.27	127.18±34.66	-0.345	0.731

*TC = Total Cholesterol; *Significant at p<0.05*

DISCUSSION

Nigeria is a third world developing country with Low Gross Domestic Product and larger percentage suffering from different forms of malnutrition due to abject poverty. Nutrition has attracted an extensive attention in various clinical fields. Generally, malnutrition is considered as an indicator related to increased morbidity and mortality (Vandewoude *et al*., 2019), therefore an assessment of nutritional state of Nigeria COVID-19 patients is necessary. The early cases of COVID-19 patients in Ibadan, Nigeria presented mostly with mild conditions, and this was related to non-fatal nature of SARS-CoV-2 strain in Nigeria at that time among other factors (Arinola *et al*., 2021).

A significant decrease was observed in prealbumin levels of COVID-19 patients when compared with un-infected healthy control. Inflammation due to cytokine storm and oxidative stress are present in COVID-19 patients (Cecchini and Cecchini, 2020). A study had reported inflammation as an effective inhibitor of protein synthesis, which may inhibit the synthesis of prealbumin (Keller, 2019). Thus, low levels of prealbumin are expected in COVID-19 patients as reported in this study. Also, prealbumin clears circulating toxic metabolites, toxic oxygen species and viral metabolites are known to be in high levels in COVID-19 patients (Wu *et al*., 2020). Thus, consumption of prealbumin in order to neutralize circulating soluble toxic matters might explain its low level in COVID-19 patients compared with controls.

Mean albumin level in COVID-19 patients compared with un-infected healthy control was non-significant compared with control. This is in tandem with previous findings that the level of albumin in COVID-19 patients was normal (Haiyan *et al*., 2020; Liu *et al*., 2020). It was reported that the Angiotensin Converting Enzymes 2 expression in human hepatocytes is not as high as in other organs (Hamming *et al*., 2004), which might explain the non-significant influence of SARS-CoV-2 infection on the synthesis of albumin by the liver.

Table 7:
CONUT distributions of COVID-19 patients according to age, gender and days of admission.

		CONUT distributions					
Variable	**Category**	**Normal**	**Moderate**	**Mild**	**Severe**	**χ^2-values**	**p – values**
Gender	Males	24 (35.8)	0 (0)	43 (64.2)	0 (0)	0.043	0.835
	Females	11 (39.3)	0 (0)	17 (60.7)	0 (0)		
Age	<40 years	19 (27.5)	0 (0)	50 (72.5)	0 (0)	1.173	0.279
	≥40 years	10 (38.5)	0 (0)	16 (61.5)	0 (0)		
DOA	≤10 days	17 (30)	0 (0)	40 (70)	0 (0)	0.464	0.496
	>10 days	11 (29)	0 (0)	27 (71)	0 (0)		

DOA = Days of admission*Significant at p<0.05

Table 8:
PNI distributions of COVID-19Patients according to Age, gender and days of admission

		PNI distributions				
Variable	**Category**	**Normal**	**Moderate**	**Severe**	**χ^2-values**	**P – values**
Gender	Males	54 (80.6)	13 (19.4)	0 (0)	0.044	0.833
	Females	23 (82.1)	5 (17.9)	0 (0)		
Age	<40 years	53 (76.8)	16 (23.2)	0 (0)	0.415	0.519
	≥40 years	21 (80.8)	5 (19.2)	0 (0)		
DOA	≤10 days	44 (77.2)	13 (22.8)	0 (0)	1.856	0.173
	>10 days	32 (84.2)	6 (15.8)	0 (0)		

DOA: Days of Admission**;** *Significant at p<0.05

Table 9:
Pearson's correlation coefficients between age, days of admission with nutritional markers or indices in COVID-19 patients.

Correlating pairs		R	P – value
Age	ALB	-0.054	0.697
	PAB	0.016	0.906
	TC	0.228	0.101
	PNI	-0.046	0.740
	CONUT	-0.118	0.405
DOA	ALB	0.226	0.097
	PAB	0.108	0.430
	TC	0.026	0.854
	PNI	0.251	0.067
	CONUT	-0.006	0.967

Abbreviations: DOA = Days of Admission, ALB = Albumin, PAB = Prealbumin, TC = Total Cholesterol, CONUT: Controlling Nutritional Status Score, PNI: Prognostic Nutritional Index
*Significant at $p<0.05$

A significant decrease in total cholesterol levels of COVID-19 patients was observed when compared with healthy un-infected subjects. Previous authors reported abnormal lipid metabolism in COVID-19 patients (Cao *et al*., 2020; Fan *et al*., 2020). Our finding might be attributed to alteration of vascular permeability by SAR-CoV-2 leading to leakage of cholesterol molecules into tissues, such as alveolar spaces forming exudates. Also, low mean cholesterol level in COVID-19 patients might due to high degradation of lipids because of its vulnerability to free radicals generated by viral infected host cells. High concentrations of free radicals were reported in COVID-19 patients (Arinola, 2020). Reduced cholesterol level in COVID-19 patients considered for this study might also be due to its consumption since it was shown that cholesterol enhances viral entry, increases ACE 2 and furin availability (Wang *et al*, 2020). Therefore, cholesterol might have been used up during SARS-CoV-2 infectivity, therefore the use of cholesterol-lowering treatment may worsen the outcome of a COVID-19 infection as previously pointed out (Ravnskov, 2020; Guirgis *et al*., 2020).

Albumin shows a significant increase in COVID-19 patients who spent more than 10 days on admission compared to those that spent less than or equal to 10 days**.** This may be related to protein rich diet given to COVID-19 patients or evidence of reduced inflammation since albumin is a negative acute phase reactant. Increased total cholesterol or albumin reported at discharge of COVID-19 patients who spent >10days on admission might indicate patients' recovery because Fan *et al*., (2020) observed that the total cholesterol levels in COVID-19 patients showed significant decreases at the time of admission as compared to the levels prior to infection, remained relatively low during the course of treatment and returned to the levels prior to infection by the time of discharge.

In this study, Controlling Nutritional (CONUT) status and Prognostic Nutritional Index (PNI) were assessed as markers of nutritional status in COVID-19 patients. Based on CONUT and PNI score of COVID-19 patients, a larger percentage (75.6%) of COVID-19 patients at admission had a mild CONUT score, 24.4% had normal CONUT score while a larger percentage (90%) were classified to have a normal PNI score indicating a well-nourished state of COVID-19 patients. Normal to mild nutritional scores in our COVID-19 patients especially in female patients and those less than 40 years might predict reduced length of hospitalization because poor nutritional state was found to be linked with delay recovery and the protraction of the hospitalization (Van Tonder *et al*., 2017). This present finding confirmed earlier report that most cases of COVID-19 in an Infectious Disease Center, Olodo, Ibadan, Nigeria were mostly mild (Arinola *et al*., 2021).

This study concludes that PNI and CONUT could be use as cheap, reliable and affordable nutritional prognostic tools in the management of COVID-19 patients.

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Full length Research Article

Impact of the COVID-19 Pandemic on Histopathological Diagnosis of Breast Tumours in Calabar, Nigeria

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Summary: The Coronavirus-19 transmitted through physical contact, droplets, and fomites caused severe respiratory disease resulting in high mortality worldwide. The COVID-19 pandemic caused innumerable hardships, panic, and restrictions of movement which negatively affected the assessment of healthcare services like breast cancer diagnosis in many countries. The results from the histopathological diagnosis of breast tumours have been routinely employed for the treatment and management of these diseases. This study investigated the impact of the COVID-19 pandemic on the histopathological diagnosis of breast tumours in Calabar. A retrospective study of the newly diagnosed breast tumours recorded in the Histopathology Laboratory register during the COVID-19 and the post-COVID-19 recovery from January 2020-February 2021 was compared with cases diagnosed before the pandemic from January 2018 to February 2019. Descriptive and inferential statistics and the Artificial Neural Network (ANN) of Statistical Package for Social Sciences (SPSS) were used for data analysis. New breast tumours diagnosed based on month showed low rates of 2.4% and 1.2% during the first and second waves of the pandemic respectively. The diagnosed cases increased to 11.8% and 8.2% after the first and second waves of the virus respectively. There was a significantly strong negative correlation between the COVID-19 pandemic and lockdown measures with breast tumour diagnosis (r=-0.919, p=0.001). More benign tumours of 56(58.3%) cases with a mean age of 25.3±11.1 years were recorded before the pandemic and were statistically significant (F=64.260, p=0.004). More malignant cases of 48(57.1%) with a mean age of 47.5±11.7 years were recorded during the pandemic. The diagnosis of malignant tumours was statistically significant between both periods (F=183.550, p=0.001). The ANN model predicted a 25% reduction in breast tumour diagnosis during the pandemic. There was a 100% impact of the pandemic on tumour type, the nature of specimen, and mean age of subjects. The COVID-19 pandemic disrupted the assessment of healthcare services as a smaller number of women were diagnosed with breast tumours during the period. This may have caused delays and late presentation leading to the diagnosis of more malignant tumours. There is a need to put adequate measures to encourage the assessment of diagnostic services during pandemics as delays may lead to an increase in morbidity and mortality.

Keywords*: COVID-19 pandemic; breast tumours; malignant; benign; diagnosis; artificial neural networks*

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INTRODUCTION

Early detection and treatment of breast cancer are vital for better management and survival (BSWG *et al*., 2021). Research has shown that the COVID-19 pandemic affected and caused disruptions in the assessment of breast cancer diagnosis, screening, and other healthcare services in most countries (BSWG *et al*., 2021; Vrdoljak *et al*., 2021). The COVID-19 virus was first reported in December 2019 in Wuhan, China (WHO, 2020a). The COVID-19 virus is a coronavirus that causes severe respiratory disease and is transmitted by physical contact, droplets, and fomites with a high rate of mortality (WHO, 2020b).

1n 2020, COVID-19 spread worldwide and the index case in Nigeria was reported on 27th February 2020 while another confirmed case was reported on 9th March 2020 (NCDC, 2020a). The outbreak of COVID-19 in Nigeria led to lockdown measures by the government by the end of March 2020 restricting movement and causing a scare in physical contact with persons. Healthcare services were still rendered but witnessed a poor turnout of patients. In Calabar, Cross River State, although, there was no report of confirmed COVID-19 cases until November 2020, when 87 new Covid-19 cases were reported in Cross River State (NCDC, 2020b), there was a poor turnout of patients for histopathological diagnosis.

The prevalence of breast cancer in Calabar is increasing and worrisome as most occur among women below 50 years of age (Ebughe *et al*., 2016; Udonkang *et al*., 2021a). Also of concern is the fact that most women present late for histopathological diagnosis. This has led to an increase in morbidity and mortality from the disease in the study area

(Ebughe *et al*., 2016). It is therefore pertinent to state that any negative condition such as the COVID-19 pandemic that puts a challenge in the assessment of diagnostic services further delays disease diagnosis for the affected persons.
As such, adequate healthcare measures to encourage the assessment of diagnostic services are paramount even during pandemics to avoid delay and reduce the associated morbidity and mortality. To date, there is a paucity of data about the effect of the pandemic on breast cancer diagnosis in Calabar. This study assessed the effect of the COVID-19 pandemic and lockdown measures on the histopathological diagnosis of breast tumours in Calabar.

MATERIALS AND METHODS

Study design/subjects/data collection: This was a retrospective study in a tertiary hospital-based cancer diagnostic center. Data from the pre-COVID-19 period of January 2018 to February 2019 and the COVID-19 period from January 2020 to February 2021 were used. All clinical data and histopathological reports from 180 female subjects aged 13-84 years were retrieved from the register of the Histopathology Laboratory, University of Calabar Teaching Hospital, Calabar. Data included the age of the subject, the nature of the tissue, laterality, and histopathological diagnosis. Histopathological diagnosis of breast tumours was based on the Scarff-Bloom-Richardson tumour grading system with Haematoxylin and eosin-stained sections.

Ethical approval and study population: Ethical approval was obtained from the University of Calabar Teaching Hospital Research and Ethics Committee with approval numbers UCTH/HREC/33/694 and UCTH/HREC/33/527.

The sample size calculation of the study was based on the sample size formula of difference in two proportions (Goyal, 2013) given as Z2P1(1-P1) + P2(1-P2) /d2 where Proportions, P1=80%, P2=90%, Z is confidence interval at 95% =1.96, and d is relative precision =10%. This gave the sample size of 96 per period resulting in 192 subjects but data from 180 eligible subjects within the study period was used after appropriate sampling was done. Inclusion criteria involved adding all women who had complete clinical data and were diagnosed with breast tumour within the study period. Women with incomplete data and men were excluded.

Statistical analysis: Statistical Package for Social Sciences (SPSS) version 20 (Armonk, New York: IBM Corporation) was used to analyze the data. Descriptive statistic was used to analyze the demographic characteristics and histopathological diagnosis of the breast tumours. Pearson correlation was used to establish the association between the COVID-19 outbreak during the pandemic months and newly diagnosed breast tumours. Chi-square was used to analyze the associations among the clinical characteristics of the breast tissues of subjects. Analysis of Variance showed the association between the mean ages of the subjects and types of benign and malignant tumours between the pre-COVID-19 and the COVID-19 periods. All results were statistically significant at a probability level less than or equal to 0.05. The Multilayer Perceptron (MLP) model of artificial neural network (ANN) of SPSS version 20 was used to test the accuracy of the impact of the pandemic on breast tumour diagnosis.

Artificial neural network design: In the MLP model used to build the neural network, period (pre-COVID-19 and COVID-19) was the dependent variable. The factors (tumour type, nature of specimen, mean age) and sub-factors (benign, malignant, unclassified type, lump, biopsy, mastectomy, unclassified nature of tissue, mean age) were the independent variables. The datasets were assigned randomly to moderate training conditions into training (60%), testing (20%), and holdout (20%) layers. The training data was used for weight determination and model building. The testing data was used to find errors, and the holdout data was used for model validation. The activation function was hyperbolic tangent (tanh) for the hidden layer. The output layer used the softmax function as the activation function. The batch training option was used for training. Initial lambda was set at 0.0000005, initial sigma was at 0.0005, interval center was 0, and interval offset was ±0.5. Cross entropy served as the error function because of the softmax function.

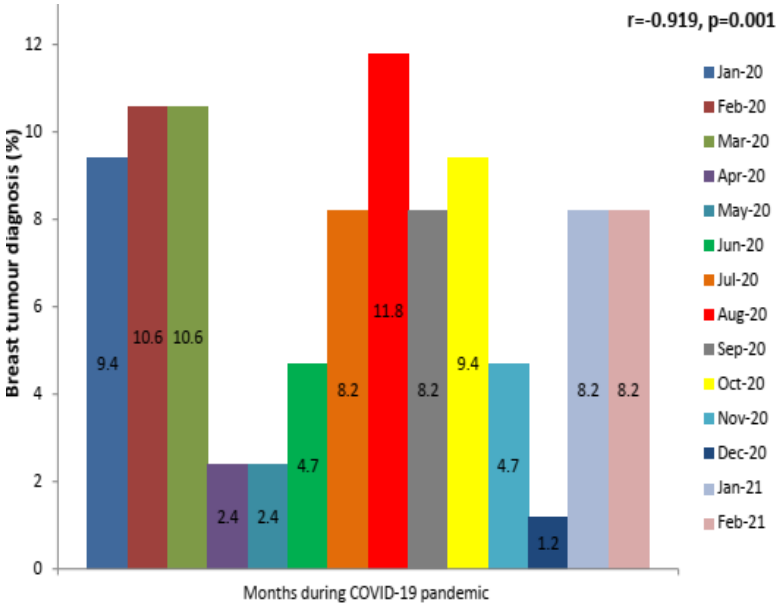

Figure 1:
The association between the Covid-19 pandemic months and newly diagnosed breast tumour

RESULTS

Fig. I is the association between the COVID-19 pandemic months and newly diagnosed breast tumours. During the pandemic, 10.6% of breast tumours were diagnosed in February and March 2020 before the lockdown. During the first wave of the pandemic and the lockdown of April and May 2020, 2.4% of cases were reported respectively. When the lockdown was lifted in June 2020, the cases increased gradually to a peak of 11.8% in August 2020. There was another decrease from 4.7% in November 2020 to 1.2% of cases in December 2020 during the second wave of the virus. However, post-COVID-19 increases in diagnosed cases were observed in January and February 2021 with 8.2% of cases respectively. There was a significantly strong negative correlation between the COVID-19 pandemic and new breast tumour diagnosis (r=-0.919, p=0.001).

In Table 1, the clinical characteristics of the breast tissues of the subjects are shown. The characteristics of subjects who were diagnosed with breast tumours during the pre-COVID-19 period showed more benign cases 56(58.3%) while more malignant cases were recorded during the COVID-19 pandemic 48(57.1%) and were statistically significant ($\chi 2$=9.391, p=0.009). Unilateral breast lesions of either part were the most recorded during both periods. Breast laterality was not statistically significant ($\chi 2$=5.065, p=0.167). More lumps 55(57.9%) during the pre-COVID-19 and more biopsies 45 (52.9%) during the pandemic were used for diagnosis. The nature of the specimen was statistically significant ($\chi 2$=29.926, p=0.001). The age range of subjects was between 13-84 years. The mean ages of the subjects were 35.2±15.4 and 39.2±14.6 during the pre-COVID-19 and in the COVID-19 periods respectively but were not statistically significant ($\chi 2$=0.216, p=0.642).

Table 1:
The clinical characteristics of the breast tissues of the subjects

Parameter	Pre-COVID-19 period n (%)	COVID-19 period n (%)	Statistics
Type of tumour			
Benign	56 (58.3)	37 (17.1)	
Malignant	35 (36.5)	48 (57.1)	
Suspicious	4 (4.2)	0 (0)	**χ^2=9.391, p=0.009**
Total	95 (100.0)	85 (100.0)	
Breast laterality			
Right	37(38.9)	35(38.5)	
Both	7(7.4)	1 (7.3)	**χ^2 =5.065, p=0.167**
Left	35(36.8)	38 (37.5)	
Unclassified	16(16.8)	11 (16.7)	
Total	95 (100.0)	85(100.0)	
Nature of tissue			
Lump	55 (57.9)	28 (32.9)	**χ^2=29.926, p=0.001**
Biopsy	22 (23.1)	45 (52.9)	
Mastectomy	15(15.8)	2 (2.3)	
Unclassified	3(3.2)	10 (11.8)	
Total	95 (100.0)	85 (100.0)	
Mean age (years)	35.2±15.4	39.2±14.6	**χ^2=0.216, p=0.642**

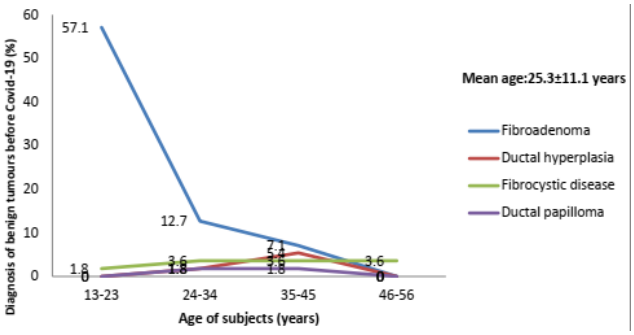

Figure 2:
The association between age and types of benign tumours during the pre-COVID-19 period.

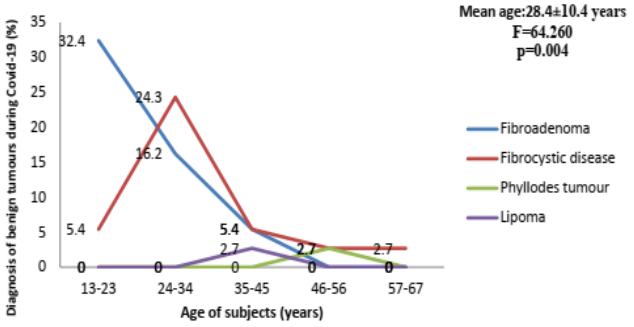

Figure 3:
The association between age and types of benign tumours during the COVID-19 pandemic.

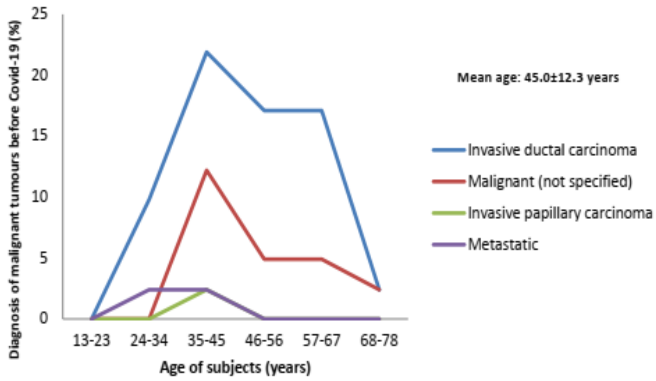

Figure 4:
The Association between age and types of malignant tumours during the pre-COVID-19 period.

The association between age and types of benign tumours during the pre-COVID-19 period is shown in Fig. 2. Among the benign tumours, fibroadenoma was the most diagnosed 43(76.8%) with a mean age of 25.3±11.1 and the majority 32(57.1%) recorded in 13-23 years during the pre-Covid period. The association between age and types of benign tumours in the COVID-19 period is shown in Fig. 3. Less fibroadenoma 20(54.1%) were diagnosed with a mean age of 28.4±10.4 years and the majority in the age range 13-23 years. There was a statistical difference in the diagnosis of benign tumours between the pre-COVID-19 and the COVID-19 periods (F=64.260, p=0.004).

Fig.4 is the association between age and types of malignant tumours during the pre-COVID-19 period. Among the malignant tumours, more invasive ductal carcinomas (IDC) 28(68.3%) with a mean age of 45.0±12.3 and peak occurrence of 9(21.9%) at 35-45 years were diagnosed. Fig.5 is the association between age and types of

malignant tumours during the COVID-19 period. During the pandemic, 31(64.6%) cases with a mean age of 47.5±11.7 years and peak occurrence of 15(31.3) in 35-45 years were diagnosed. The diagnosis of malignant tumours was statistically significant between both periods (F=183.550, p=0.001).

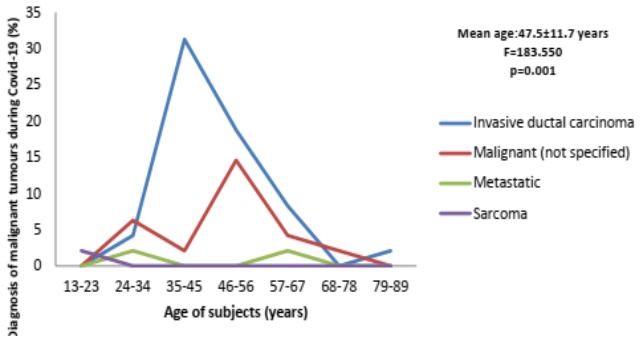

Figure 5:
The association between age and types of malignant tumours during the COVID-19 pandemic

Table 2:
Summary of MLP results for breast tumours diagnoses during both periods.

MLP RESULTS				
Parameter		N (%)	Cross entropy error	
Sample	Training	9 (64.3)	4.735	
	Testing	1 (7.1)	0.286	
	Holdout	4 (28.6)		
	Total	14 (100)		
Training time	0:00:00.02			
Classification		Pre-COVID-19	COVID -19	% Correct Prediction
Training	Overall %	56.6	44.4	66.7
Testing	Overall %	0	100	100
Holdout	Overall %	75	25	25
ROC Area	Pre-COVID-19	0.813		
	COVID-19	0.813		
Independent variable importance	Relative importance	Normalized importance (%)		
Sub-factors	0.409	69.3		
Factors	0.591	100		

Multilayer Perceptron results: The Summary of MLP results for breast cancer diagnosis during both periods is shown in Table 2. The automatic random selection assigned 9 nodes to the training layer. The testing layer had 1 node and the holdout layer had 4 nodes. The cross-entropy error for the training layer was 4.735 but reduced to 0.289 in the testing layer. This shows the error function that the network minimizes during the training phase. The small value of this error indicates the power of the model to predict the outcome. The classification results showed that the overall percent prediction for training was 66.7% (pre-COVID-19=56.6%, COVID-19=44.4%), testing was 100% (pre-COVID-19=0%, COVID-19=100%), and the hidden layer was 25% (pre-COVID-19=75%, COVID-19 =25%). The overall percent prediction for cases during the pre-COVID-19 period was 75% and reduced to 25% during the COVID-19 period. The accuracy of prediction was 25% during the COVID-19 outbreak. The areas under the Receiver Operating Characteristic (ROC) curve for the pre-COVID-19 and COVID-19 periods were 0.813 respectively. This indicates a good classification of the cases diagnosed during both periods.

The impact of the periods on the factors and sub-factors was indicated by the normalized importance. The pandemic had a 100% impact on the factors and a 69.9% impact on the sub-factors respectively. Fig. 6 is the MLP neural network of factors and sub-factors of breast tumour during both periods. The neural network shows the summary of these interactions.

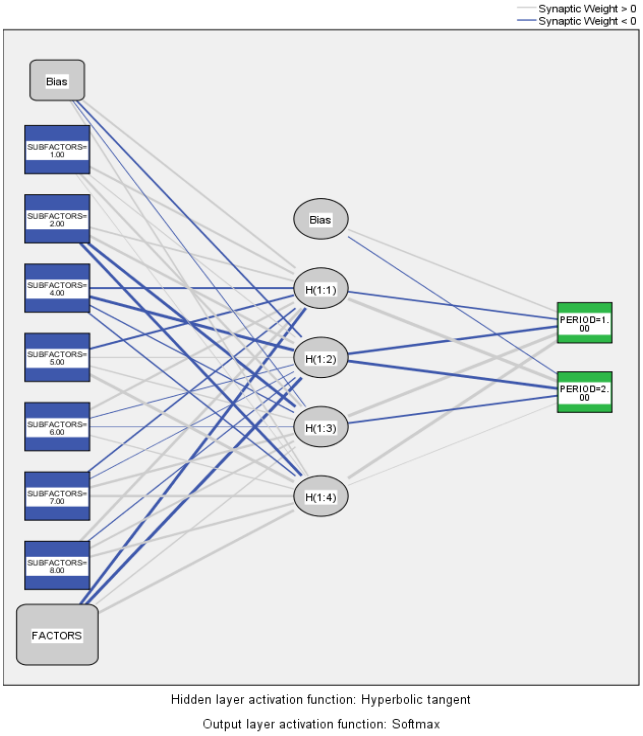

Figure 6:
The MLP neural network of factors and sub-factors of breast tumours during both periods
Keys: PERIOD(1)=pre-COVID-19, PERIOD(2)=COVID-19, FACTORS (tumour type, nature of specimen, mean age) and SUB-FACTORS (1=benign, 2=malignant, 3=unclassified-type, 4=lump, 5=biopsy, 6=mastectomy, 7=unclassified-nature of tissue, 8=mean age)

DISCUSSION

The COVID-19 pandemic and lockdown measures caused disruptions and inaccessibility to most medical services (Bosch *et al*., 2022). This research evaluated the outcome of this pandemic on the histopathological diagnosis of breast tumours. In this study, there was a reduction in the number of newly diagnosed cases of breast tumours. This may be attributed to the panic and COVID-19 lockdown measures imposed by the government during the period. Although diagnostic services were not halted there was a low turn up for assessment of these services by the public. This is similar to the report of decreased breast cancer diagnoses by 7% in 2020 (Yong *et al*., 2021).

This study reported the occurrence of breast cancers particularly invasive ductal carcinomas using mostly breast biopsies and fewer mastectomies for diagnosis during the pandemic. Invasive ductal carcinoma has been the commonest breast cancer affecting women in Calabar. This is similar to previous reports (Ebughe *et al*., 2016; Udonkang *et al*., 2021). The increase in biopsies confirms the increase in advanced disease from benign tumours that use mostly lumps for diagnosis. The fewer mastectomies indicate a few late stages of disease at diagnosis. Overall, this showed that there might have been a delay in seeking early diagnosis during the pandemic resulting in an increased risk of developing advanced disease. Delays in seeking early diagnosis have been previously reported in Calabar (Ebughe *et al*., 2019). A similar delay in diagnosis during the COVID-19 pandemic was reported in Croatia (Vrdoljak *et al*., 2021).

Another finding shows that the benign tumours affected younger women of 13-23 years in confirmation of the benign changes associated with lumps among women of this age. The invasive ductal carcinomas affected mostly women of 35-45 years in the study. This confirms that the new cases were mostly among premenopausal women below 50 years of age. New cases of breast cancer have continued to affect younger women of reproductive age as reported in previous work (Udonkang *et al*., 2021a). The effect of age has been linked to the persistent influence of reproductive hormones on breast physiology during reproductive life (Lee and Sultanian, 2015). The numerous cellular and extracellular matrix changes especially involving collagen fibres may also be aetiological to the development of cancers following the early onset of benign diseases as supported by previous work on collagen changes in breast tumours (Udonkang *et al*., 2021a; Udonkang *et al*., 2021b).

The MLP neural network gave a good prediction of the effect of the COVID-19 pandemic on the diagnosis of breast tumours. Also, the MLP model gave a good performance classification of the cases diagnosed during both periods as seen with the result of the area under the ROC curve. The model gave an accuracy of reduction in breast tumour diagnosis during the pandemic. The MLP model has been proven to give a correct classification of performance when applied (Zacharis, 2016). This is similar to the ability of MLP to predict risk factors and biomarkers in leiomyoma (Udonkang *et al*., 2022). Tumour type, the nature of specimen, and mean age were factors that were greatly impacted during the pandemic. This confirms earlier results of diagnosis of more invasive ductal carcinomas, more biopsies, and the occurrence of most diseases in younger women below 50 years (Udonkang *et al*., 2021a) in the study area.

In summary, the COVID-19 pandemic caused a decrease in the newly diagnosed cases of breast tumours in Calabar. One reason may have been the lockdown measures which negatively affected the movement of people. Most persons were as well overwhelmed by the fear of contracting the virus during visits to the hospital. Also, a change in the attitude of healthcare providers in attending to patients by adopting a case-by-case prioritization approach may have been an additional contributory factor. These factors have been stated in similar studies of the effect of COVID-19 on other healthcare services in Nigeria (Olabumuyi *et al*., 2020; Ekpenyong *et al*., 2020).

The strength of this study is based on its novelty and availability of data from the Register of the Histopathology laboratory of the women diagnosed with breast tumours and the application of neural networks in data analysis. The limitation of this study was on incomplete classification of data of some subjects from the records which lead to some exclusion.

In conclusion, the COVID-19 lockdown measures caused a reduction in the number of newly diagnosed breast tumours during the pandemic months when compared to the pre-COVID-19 and post-COVID-19 months. More benign tumours were diagnosed among younger women in their twenties while women below 50 years were mostly diagnosed with cancers. There is a need to put adequate measures to ensure unrestricted access to diagnostic services even during pandemics to reduce the morbidity and mortality associated with delay in early breast cancer diagnosis.

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Full length Research Article

Selenium and Omega-3 Fatty Acids Ameliorate Highly Active Anti-Retroviral Therapy (HAART)-induced Reproductive Impairment in Male Wistar Rats

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Summary: Highly active anti-retroviral therapy (HAART) is currently the main stay in the treatment of Human Immunodeficiency Virus (HIV) disease. This treatment regimen typically combines three or more antiretroviral drugs. Like most drug combinations or polypharmacy, HAART has side effects including those on reproductive function which could place HIV patients on HAART under double risk in terms of reproductive function. Part of tissue damage following HAART administration is blamed on oxidative stress. We therefore sought to explore effects of Omega 3 and Selenium, two common antioxidants on HAART-induced male reproductive impairment in a non-HIV animal model. Sixteen male adult Wistar rats weighing 120g to 250g used for the study were divided into 4 groups of four rats each (control, HAART-only, HAART + Omega 3 and HAART + Selenium groups). Duration of daily administration was six weeks. Results showed no significant changes in pH of epididymal semen among the groups. Sperm count and viability were significantly reduced in HAART-only compared with control ($p<0.05$) but increased in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group ($p< 0.05$). Sperm motility was significantly reduced in HAART-only compared with control group ($p< 0.05$). A significantly higher percentage of total sperm defects was observed in HAART-only group compared with control ($p <0.05$) but significantly lower in the HAART + Selenium compared with HAART-only groups ($p<0.05$). Serum testosterone was significantly reduced in HAART-only compared with control groups ($p<0.05$) but significantly increased in HAART + Omega 3 and HAART + Selenium groups compared with HAART- only group ($p<0.05$). Serum concentration of luteinizing and follicle stimulating hormones were not significantly different among the groups. Testicular concentration of malondialdehyde was significantly increased in HAART-only compared with control ($p<0.05$) but significantly reduced in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group ($p<0.05$ in each). Testicular glutathione peroxidase activity was significantly reduced in HAART-only and HAART + Selenium groups compared with control ($p< 0.05$), but significantly higher in HAART + Omega 3 compared with HAART-only groups ($p<0.05$ each). Testicular superoxide dismutase activity was significantly lower in the HAART-only and HAART + Selenium compared with control ($p<0.05$) but significantly higher in HAART + Omega 3 and HAART + Selenium compared with HAART-only groups ($p<0.05$ each). Level of tumour necrosis factor – alpha in testes was significantly higher in HAART-only ($p<0.05$) compared with control but lower in the HAART + Selenium and HAART + Omega 3 ($p<0.05$) groups compared with HAART-only groups. It was also significantly lower in HAART + Selenium compared with control ($p<0.05$ each) groups. Interleukin-6 levels were significantly increased in all HAART-administered groups compared with control ($p<0.05$ each) though significantly lower in HAART + Omega 3 and HAART + Selenium compared with HAART-only groups ($p<0.05$ each). In conclusion, co-administration of Omega 3 or Selenium with HAART ameliorates HAART-induced male reproductive impairment as well as alterations in redox and inflammatory status in male rats.

Keywords: *HAART, male reproductive impairment, Omega 3, Selenium*

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INTRODUCTION

Human immunodeficiency virus (HIV) infection and AIDS are associated with systemic affectations in virtually every body system and majorly indirectly (Kibaru *et al*, 2015, WHO 2021). This pandemic has sub-Saharan region of Africa being worse affected having over two thirds of the world's HIV infection cases (WHO, 2021; Kalling, 2008; UNAIDS 2007). The reproductive system is not spared in the complications of HIV infection (Kushnir and Lewis, 2011).

The effect of HIV infection on the reproductive system could be due to biological changes like weight loss, systemic illnesses, and stress (Khawcharoenpom and Sha, 2016). The effect on the reproductive system could also be due to AIDS-related co-morbidities like orchitis, epididymitis, pelvic inflammatory disease as well as co-infection with

sexually transmitted pathogens and from opportunistic microbes (Bezold *et al*, 2007). Infection with HIV has also been linked with hypogonadism which could be primary or secondary (Raffi *et al*, 1991).

The advent of highly active antiretroviral therapy (HAART) or combination antiretroviral therapy (cART) was therefore great news with potential to improve management of HIV and its complications including those on reproductive function. This drug treatment regimen or cocktail contains at least three drugs from two or more classes of antiretrovirals given in combination (Mulata *et al*, 2015; Oyeyipo *et al*, 2018). The introduction of HAART therefore heralded an important gain in the fight against HIV/AIDS as the combination therapy targets different stages in the life cycle of the Human Immunodeficiency Virus and so reducing the tendency for drug resistance.

However, like most drugs, drug combinations or polypharmacy, the administration of HAART has been associated with several unwanted effects including those on reproductive function. These effects could be a primary effect of a particular drug component or a synergistic effect of two or more drugs in the cocktail. Administration of HAART has been associated with gonadotoxicity in male rats (Osonuga *et al*, 2010; Bakere *et al*, 2020) as well as impaired sperm motility (Oyeyipo *et al*, 2018; Akhigbe *et al*, 2021; Akang, *et al*, 2022; Savasi *et al*, 2019). Other effects on the reproductive system induced by HAART include reduced serum testosterone, reduced semen volume and impaired sperm count, viability and morphology (Akhigbe *et al*, 2021, Kushnir and Lewis, 2011). Other complications of HAART are sperm mitochondrial deletion (White *et al*, 2001) as well as increased sperm DNA fragmentation (Akang *et al*., 2020, Savasi *et al*, 2018). Oxidative stress has also been noted in testes of patients treated with HAART (Akang *et al*, 2022; Oyeyipo *et al*., 2018) and this can be measured either directly by evaluatng the products of processes like malondialdehyde (MDA), hydrogen peroxide, or indirectly by assessing the concentration or activities of antioxidant systems like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) etc. (Marrocco *et al*, 2017; Yoshikawa and Naito, 2002).

From the above literature, one can observe that though the introduction of HAART has great potential to reduce morbidities and mortalities associated with HIV infection, it is not without its own side effects including those on the reproductive function, the pathophysiology of which is partly attributed to oxidative stress (Akang *et al*., 2022; Oyeyipo *et al* 2018). It was based on this that the possible effects of Omega 3 and Selenium, two commonly available and cheap antioxidants on HAART-induced reproductive toxicity were evaluated.

MATERIALS AND METHODS

Ethical Approval: Ethical consent with approval number 108PHY3821, was granted by the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar – Nigeria.

Drugs: A combined antiretroviral therapy (cART) drug (Mylan Laboratories Ltd, India) composed of 50mg Dolutegravir-DTG (as dolutegravir sodium), 300mLamivudine-3TC and 300mg Tenovir Disoproxil Fumarate-TDF per tablet was obtained from the Antiretroviral Unit of Infections Disease Hospital (IDH), Calabar, Cross River State, Nigeria. Omega 3 fatty acid (Emzor Omega-3® fish oil, 1000mg per capsule) manufactured by Gujarat Liqui Pharmacaps Pvt Ltd – GLPL, India) containing Eicosapentaenoic Acid NLT (18%), Docosahexaenoic Acid NLT (12%) and Selenium as Sodium Selenite (200mcg per capsule), made by Bactolac Pharmaceutical Inc. USA were obtained from Bez Pharmacy, Eta Agbor Road, Calabar.

Extrapolation of HAART dosage: The dosage for rats was extrapolated from human doses using the formular developed by Nair and Jacob (2016). Animal equivalent dose – AED (mg/kg, Human dose (mg/kg) x Kin ratio

Where (Km) = corrected factor estimated by dividing the average body weight (kg) of species to its body surface area (m2).

Preparation of drugs/stock solutions/dosages: Tween-80\water solution: Ten drops of the solvent (Tween-80) were placed on a graduated bottle and made up to 40mls with portable water.

Stock solution of HAART. This was prepared by reducing two tablets of HAART to powder, then dissolving it in 8 drops of Tween-80 and the resulting solution made up to 40mls with water to give concentrations of 2.5mg, 15mg and 15mg of Dolutegravir, Lamivudine and Tenovir Disoproxil Fumarate respectively per ml of solution.

Stock solution of Omega 3: This was done by dissolving the content of six capsules of Omega 3 (6000 mg) in 4 drops of Tween-80 and the solution marked up to 40mls giving a concentration of 150mg/ml of solution.

Stock solution of Selenium: This was prepared by dissolving the content of 10 capsules (2000µg) in 4 drops of Tween-80 and the solution marked up to 40mls to give a concentration of 50µg/ml of solution.

Experimental Design: Sixteen male Wistar rats weighing 120 – 250g were randomly divided into four groups of 4 rats per group and raised in wooden cages which were cleaned regularly. Animals were given food and water ad libitum. Group 1 (control) was given 0.003ml/g of the Tween-80/water solution daily. Group 2 (HAART-only) was given 25mg/kg, 25mg/kg and 3.8mg/kg (ie 0.00167ml/g) of Lamivudine, Tenovir and Dolutegravir respectively of the HAART solution. Group 3 (HAART + Omega 3) received 0.00167ml/g (i.e. 3TC-25mg/kg, TDF-25mg/kg and DTG-3.8mg/kg) of HAART solution + 600mg/kg of Omega 3. Rats in group 4 (HAART + Selenium) received 0.00167ml/g (3TC-25mg/kg, TDF-25mg/kg and DTG-3.8mg/kg) + 0.3mg/kg (0.0075ml/g) of Selenium. Drugs were administered daily by gavaging for duration of six (6) weeks. Reweighing of animals was done on a regular basis to take care of needed adjustments in the amount of drugs administered.

Collection of samples: At the end of administration period (six weeks) animals were anaesthesized under chloroform and blood samples collected via intracardiac puncture for

assay of relevant parameters. Animals were then sacrificed and testes dissected out for determination of oxidative stress, seminal and inflammatory parameters.

Seminal fluid analysis: Epididymal seminal fluid analysis was carried out following standard procedures outlined by the World Health Organization WHO (2006) summarized below:

Seminal Ph: Semen was aspirated from epididymis with a sterile needle and the pH measured directly using a hand-held pH meter (Zellik, Belgium)

Sperm count: This was done using improved Neubeur counting chamber. A sample of 0.5ml of sperm suspension was diluted with 9.5ml of sperm-diluting solution (5g NaHCO3, 1ml formalin (35%) and 25mg Eosin per 100ml distilled water). Ten microlitre (10µl) of the diluted sperm suspension was transferred to each counting chamber and allowed to stand for 5 minutes. The concentration of sperms was evaluated as million sperm cells per ml of sperm solution under x 400 magnification using light microscope (Olympus, Tokyo, Japan).

Sperm viability: One (1) drop of semen was mixed with one (1) drop of 0.5% Eosin solution on a slide. The preparation was left for two (2) minutes after which x10 objective was used to focus the specimen while x40 objective was used to evaluate the per centage of viable (unstained) and nonviable (stained) sperm cells.

Sperm morphology: A thin smear of well mixed semen was made on a slide and while still wet fixed with 95% v/v ethanol for five (5) minutes and allowed to air-dry. The smear was then covered with carbol Fushcin (1 in 20), allowed to stand for 30mins and then washed off with water. It was then counter-stained with diluted Leoffler methylene blue for two (2) minutes. Using x40 objective lens, morphology of sperm cells was assessed and expressed in percentage.

Sperm motility: One (1) drop of well mixed semen was placed on a slide and covered with a cover glass. A light microscope (Olympus, Tokyo, Japan) was then used to focus the specimen using x10 objective lens. A x40 objective lens was thereafter used to examine several fields for motility with results expressed in percentage (%).

Determination of serum concentration of follicle stimulating hormone (FSH): This was determined in triplicated samples by Chemiluminescent Microparticle Immunoassay (CMIA) technique using ARCHITECT FSH Kit (7K75) obtained from Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, USA- and as used by Tan *et al* (2018).

Evaluation of serum concentration of luteinizing hormone (LH): This was done using Chemilumiscent Microparticle Immunoassay using ARCHITECH LH Kit (6C25) obtained from Abbott Laboratories Diagnostics Division, Abbott Park, IL USA. This evaluation was in tandem with similar method as demonsrated by Steyn *et al* (2013).

Determination of serum testosterone concentration: This was done using the Chemiluminescent Microparticle Immunoassay (CMIA) ELISA technique with ARCHITECT Testosterone Reagent kit obtained from Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA as demonstrated by Steyn *et al* (2013).

Preparation of testicular homogenate: Dissected testes were cleaned of fat and homogenized in 25ml saline-merthiolate-triton (SMT) buffer at 27,000 rpm using a Brinkmann Kinematical homogenizer for 2 minutes and used immediately for assay of various testicular parameters. This was as also demonstrated by Rodriguez-Casuriaga *et al* (2013).

Determination of malondialdehyde (MDA) concentration in testicular homogenate: This was determined using the method of Buege and Aust (1978). A sample of 0.1ml homogenate (in Tris-HCL buffer (at pH 7.5) was treated with 2ml (in a ratio 1:1:1) of thiobarbituric acid (TBA) – Trichloroacetic acid (TCA) and HCL reagent (TBA 0.37%, 25 NHCL and 15% TCA) after which the mixture was placed in a water bath for 15 minutes and allowed to cool. The absorbance of the clear supernatant was measured against reference blank at 535nm with a Perkin Elmer Spectrophotometer, (Lamber 25).

Evaluation of testicular glutathione peroxidase activity: This was assessed on testicular homogenate according to an established procedure of Weydert and Cullen (2010) using hydrogen peroxide (H2O2) as substrate. The absorbance of the product was read at 430nm.

Determination of testicular superoxide dismutase (SOD) activity: This was doneon testicular homogenate using biochemical method with xanthine oxidase used to generate superoxide anion (O2-) while nitroblue tetraolium (NBT) reduction was used as indicator of the O2 production. The method of Weydert and Cullen (2010) was used for this procedure. The optical density (OD) of the product was read at 450nm using a ELISA reader within 15 minutes after adding stop solution.

Evaluation of IL- 6 and TNF-alpha: The concentrations of IL-6 and TNF-alpha in testicular homogenate were determined using commercially available indirect sandwich ELISA kits (Bender MedSystems, Austria) for each as also done by Afshari *et al* (2005). At the end of reaction time, the absorbance for each was measured at 450nm. Analyses for each parameter were performed in duplicates following manufacturer's guidelines.

Statistical analysis: Data were presented as mean ± SEM. Normally distributed data were analyzed using analysis of variance (ANOVA) followed with a post hoc test of least significant differences. A value of $p<0.05$ was considered statistically significant. All analyses were conducted using Statistical Package for the Social Sciences (SPSS) software version 25.

RESULTS

Semen pH: There were no significant differences in the pH of semen in various experimental groups as in Table 1.
Sperm count: (million/ml): Sperm count was significantly reduced in the HAART-only group (34.18 ± 7.43) compared with control (55.95 ± 5.17) ($p<0.05$) but significantly higher in HAART + Omega 3 (48.15 ± 8.54) and HAART + Selenium (48.38 ± 5.14) compared to HAART-only (34.18 ± 7,43) groups ($p<0.05$ each) as shown in Table 1.

Sperm viability (%): Viability of sperms was significantly reduced in the HAART-only (32.5±2.89) compared with control (87.5 ± 14.) with $p<0.05$ but increased in the HAART + Omega 3 (83.45±6.29) and HAART + Selenium (90.00 ± 4.08) groups with $p<0.05$ as shown in Table 1.

Sperm motility (%): Sperm motility was significantly reduced in the HAART-only group (69 ± 8.21) compared with control group (86±4.9) with $p<0.05$ as shown in Table 1.
Morphological defects (%:) Percentage of total morphologically defective sperm cells was significantly higher in the HAART-only (7.5±1.29) compared with control (4 ±0.82) with $p<0.05$ but lower in the HAART + Selenium (4.75±.171) compared with HAART-only (7.5±1.29) groups with $p<0.05$ as in Table 1.

Serum FSH (mlu/ml): Comparison of the concentrations of serum FSH of control (9.27± 0.66) HAART-only (9.12±0.29), HAART + Omega 3 (8.73±0.58) and HAART + Selenium (8.81±0.35) did not show any significant differences among the groups as shown in Table 2.

Serum LH (mlu/ml): Comparison of concentrations of serum LH in control (7.45±1.17) and HAART-only (7.55±0.68), HAART + Omega 3 (7.36±0.75) and HAART + Selenium (6.67±0.12) groups did not show any significant differences among the groups as in Table 2.

Serum testosterone (ng/ml): Concentration of serum testosterone was significantly lower in the HAART-only (2.29±0.85) compared with control (4.94±0.78) with $p<0.05$ but higher in the HAART + Omega 3 (4.11±0.55) and HAART + Selenium (4.20±0.60) compared with HAART-only (2.29±0.85) groups with $p<0.05$ each as in Table 2.

Testicular concentration of malondialdehyde (nmol/ng): Concentration of testicular malondialdehyde (MDA) was significantly increased in the HAART-only (65.53±3.53) compared with control (47.89±3.28) with $p<0.05$ but decreased in HAART + Omega 3 (43.20±3.74) and HAART + Selenium (47.59±3.72) with $p<0.05$ compared with HAART-only groups as in Table 3.

Testicular glutathione peroxidase – GPx activity (pg/mg protein): Glutathione peroxidase activity was significantly reduced in the HAART-only (28.94± 2.56) compared with control (43.60±4.81) with $p<0.05$ but higher in HAART + 0mega 3 (47.17±2.88) and HAART + Selenium (35.21±4.31) when compared with HAART-only (28.94±2.56) with $p<0.05$ each. It was also significantly lower in HAART + Selenium compared with HAART + Omega 3 groups ($p<0.05$) as shown in Table 3.

Testicular superoxide dismutase activity (pg/mg protein): Superoxide dismutase activity in HAART-only (57.23±2.19) was significantly decreased compared with control (110.61±8.27) with $p<0.05$ but higher in HAART + Omega 3 (97.67±7.58) and HAART +Selenium (84.06±7.19) compared with HAART-only (57.23±2.19) groups with $p<0.05$ in each case. It was also significantly lower in HAART + Selenium (84.06±7.19) compared with control ($p<0.05$) as shown in Table 3.

Table 1:
Sperm quality parameters of the different experimental groups

Group	pH	Sperm count ($x10^6$cells/mL)	Motile sperm (%)	Viable sperm (%)	Total sperm defects (%)
Control	6.70±0.24	55.95±5.17	86.00±4.97	87.50±6.45	4.00±0.82
Haart	7.05±0.19	34.18±7.43[a]	69.00±8.21[a]	32.50±2.89[a]	7.50±1.29[a]
Haart + Omega 3	6.83±0.25	48.15±8.54[b]	80.00±9.13[b]	83.75±6.29[b]	5.00±0.82
Haart + Selenium	6.98±0.17	48.38±5.14[b]	82.50±8.66[b]	90.00±4.08[b]	4.75±1.71[b]

Values are expressed as mean ±SD; a = p<0.05 vs control; b = p<0.05 vs Haart

Table 2:
Reproductive hormones concentration in the different experimental groups

Group	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)
Control	9.27±0.66	7.45±1.17	4.94±0.78
Haart	9.12±0.29	7.55±0.68	2.29±0.85a
Haart + Omega 3	8.73±0.58	7.36±0.75	4.11±0.55b
Haart + Selenium	8.81±0.35	6.67±1.12	4.20±0.60b

Values are expressed as mean ±SD
a = p<0.05 vs control
b = p<0.05 vs Haart

Table 3:
MDA, GPx and SOD concentration in the different experimental groups

Group	MDA (nmol/mg Protein)	GPx (pg/mg Protein)	SOD (pg/mg Protein)
Control	47.89±3.28	43.60±4.81	110.61±8.27
Haart	65.57±3.53[a]	28.94±2.56[a]	57.23±2.19[a]
Haart+Omega 3	43.20±3.74[b]	47.17±2.88[b]	97.67±7.58[b]
Haart+Selenium	47.59±3.72[b]	35.21±4.31[a,c]	84.06±7.19[a,b]

Values are expressed as mean ±SD
a = p<0.05 vs control; b = p<0.05 vs Haart
c = p< 0.05 vs Haart + Omega-3

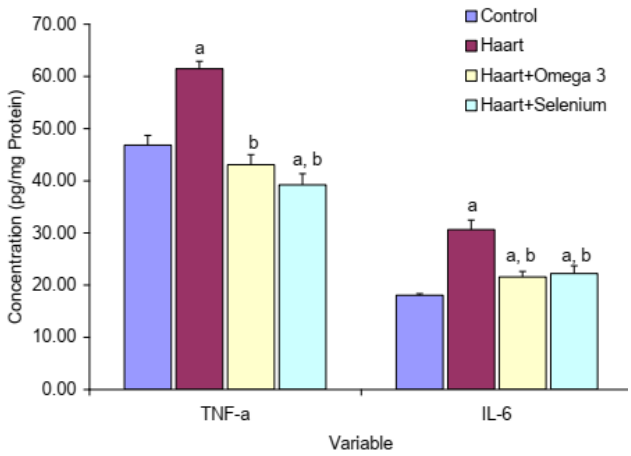

Figure 1
Tissue necrosis factor-alpha and interleukin-6 activity in the different experimental groups.
Values are expressed as mean±SD, n=5
a = p<0.05 control; b = p<0.05 HAART

Testicular concentration of tumor necrosis factor – alpha (pg/mg protein): Tumor necrosis factor-alpha (TNF-α) was significantly increased (p<0.05) in HAART-only (61.45±1.44) compared with control (46.83±1.86) but significantly decreased (p<0.05 each) in HAART + Omega 3 (43.05±1.93) and HAART + Selenium (39.20±2.16) groups compared with HAART-only group. There was also significant decrease (p<0.05) in this index in HAART + Selenium compared with control groups as shown in Fig. 1

Concentration of testicular interleukins-6 (pg/mg protein): There were significant increases (p<0.05 each) in the concentrations of interleukin-6 (IL-6) in HAART-only (30.64±1.83), HAART + Selenium (22.21±1.51) groups compared with control (18.05±0.32). it was however significantly decreased in HAART + Omega 3 and HAART + Selenium groups (p<0.05 for each) compared with HAART-only group as shown in Fig. 1.

DISCUSSION

In this study, the effects of co-administration of HAART with Omega 3 or Selenium on some male reproductive and related parameters were evaluated. pH affects the physiological functions of tissues and cells including semen (Zhou *et al*, 2010). Our results however did not show any significant differences in seminal pH among all groups indicating that any abnormality in the seminal fluid parameters might not have been due to seminal pH.

The observed significant decrease in sperm count in the HAART-only group when compared with the control strongly suggests that HAART affects sperm count. Our observation is similar to the findings by Savasi *et al* (2019), Akang *et al* (2022), Alugbe *et al* (2021), Bakare *et al* (2020), Osonuga *et al* (2010) and Oyeyipo *et al* (2018)). The decrease in sperm count could have been due to direct testicular toxicity from oxidative stress (Oyeyipo *et al* 2021), sperm DNA fragmentation (Savasi *et al*, 2018; Osonuga *et al*, 2010; Bakare *et al*, 2020) or HAART-induced sperm mitochondrial DNA fragmentation and deletion (White *et al*, 2001). The significantly higher sperm count in the groups co-administered with antioxidants (HAART + Omega 3 and HAART + Selenium groups) shows the ability of the antioxidants to ameliorate the impairment induced by HAART (Oyeyipo *et al*, 2018).

Sperm viability was significantly reduced in HAART-only group compared with control. This is similar to the findings of Savasi *et al* (2019) and Akhigbe *et al* (2021). This trend was however prevented with an increase in the percentage of viable sperms following co-administration with Omega 3 or Selenium

Normal sperm morphology is essential for fertilization. We noted an increase in percentage of morphologically defective sperm cells in HAART-only group compared with control which is similar to the observations of Bakare *et al* (2020), Akhigbe *et al*, (2021) and Savasi *et al* (2019). The increase in the percentage of defective sperms in the HAART-only group might have been due to increased sperm DNA fragmentation associated with HAART administration as reported by White *et al* (2001) and Savasi *et al* (2018). There was however a significant decrease in the percentage of morphologically defective sperm cells in the HAART + Selenium compared with HAART-only groups; an effect attributable to Selenium co-administration. Selenium as a trace element is the backbone of a number of Selenium–containing compounds including Selenoproteins like glutathione peroxidase enzymes involved in several physiological activities like immune modulation redox reactions, anti-inflammatory functions and inactivation of free radical to prevent oxidative stress (Brown and Authur, 2001; Yang & Liu, 2017; Constatineacu-Aruxandei *et al*, 2018; Xie *et al*, 2020).

Our results do not show any significant differences in the serum concentrations of FSH and LH among the different groups. These hormones are produced in the anterior pituitary gland (Sembulingam and Sembulingam 2012). This means that any effect of HAART might not have affected the synthesis of these hormones.

A significant reduction in the concentration of serum testosterone observed in HAART-only group compared with control is similar to the finding of Akang *et al* (2022). Akhigbe *et al* (2021) also found a similar decrease in testicular concentration of testosterone in HAART-administered rats. This decrease could have been due to direct gonadal toxicity affecting Leydig cells (Osonuga *et al*, 2010, Bakare *et al* 2020). Our observed reduction in concentration of testosterone in HAART-only group with no significant differences in FSH and LH shows that HAART induces a secondary hypogonadism. The decrease in testosterone might also have contributed to abnormalities observed in seminal parameters mentioned earlier. Co-administration of Omega 3 or Selenium with HAART prevented this trend.

Literature shows that administration of HAART is associated with oxidative stress, an imbalance between pro-oxidative and anti-oxidative mechanisms which tilts towards oxidation (Yoslukawa and Naito, 2002; Marrocco *et al*, 2017). In a similar way, we observed that testicular concentration of MDA, one of the last products of lipid peroxidation in tissues (Ivanov *et al*, 2016; HO *et al*, 2013), was significantly increased in HAART-only group compared with control which suggests an increase in lipid

peroxidation in the former group compared with the latter.This observation is also similar to that made by Oyeyipo *et al* (2018). On the other hand, our findings of significant decreases in activities of testicular superoxide dismutase and glutathione peroxidase in the HAART-only compared with control groups is similar to those of Oyeyipo *et al*, (2018); Akang *et al* (2021); Elechi-Amadi and Briggs (2018); Havlickoval (2021) and suggests elevated oxidative activities. The increase in peroxidation and oxidation observed in HAART-only group strongly agree with previous works mentioned above that HAART induces oxidative stress in testes and so can be partly responsible for the impaired testicular function seen in this study. This is especially so as these changes were prevented following co-administration of HAART with Omega 3 or Selenium which are known antioxidants.

From our results, there was a significantly increased concentration of testicular TNF-α in HAART-only compared with control groups. Tumour necrosis factor –α is a pleiotropic cytokine (Gough and Myles, 2020) and dysregulation of its production has been associated with several pathologies including inflammation, psoriasis, inflammatory blood disease, cancers etc. (Gough and Myles 2020; Brynskov *et al*, 2002; Victor and Gottlieb, 2002). The significant decrease in TNF-α observed in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group shows ability of Omega 3 and Selenium to prevent this effect of HAART if co-administered.

Interleukin-6, a multi-functional cytokine was found to be significantly increased in the HAART-only. Its dysregulated continual synthesis plays pathological role in chronic inflammation and immunity (Kimura and Kishimoto, 2010; Tanaka *et al*, 2014; Zhang *et al*, 2010). There was however a significant decrease of this index in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group'' The observed increase in testicular TNF-alpha and IL6 in HAART-only group suggests that part of mechanism of testicular injury might have been inflammation. This was however prevented following co-administration with Omega 3 or Selenium'.

In conclusion, co-administration of Omega-3 or Selenium with HAART ameliorates HAART – induced reproductive toxicity as well as testicular inflammatory status and oxidative stress in male Wistar rats.

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Full length Research Article

Comparative Therapeutic Effect of Single/Combined Administration of Saxagliptin, Metformin and Intranasal Insulin on Dexamethasone Induced Insulin Resistance in Wistar Rat Model

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Summary: Glucocorticoids have therapeutic benefits in the management of several inflammatory and immunological disorders. Despite these medicinal effects, they have the drawback of causing metabolic disorders such as hyperglycemia, insulin resistance etc., which is known to be a key indicator of metabolic syndrome. Metabolic syndrome is a major predisposing factor to type 2 diabetes mellitus and cardiomyopathy. This study was designed to compare and evaluate the effects of saxagliptin, metformin and intranasal insulin (when used singly or in combination) on dexamethasone induced insulin resistance. Fifty-six female rats were randomly assigned into eight groups. Group 1 represented the control; Group 2 was administered with dexamethasone (1mg/kg) and served as untreated group. Other groups were administered dexamethasone(1mg/kg) and treated with singly/combinations of intranasal insulin (2IU); metformin and (40mg/kg) and saxagliptin (8mg/kg). Treatments were given for period of one week. At the end of the study, blood samples were collected for biochemical assays such as lipid profile, serum insulin, glucagon, adiponectin, glucokinase enzymes and glucose-6-phosphatase enzymes. Representative pancreases were excised for histological examination. Results showed that dexamethasone (1mg/kg) induced hyperglycemia, hyperinsulinemia, dyslipidemia, increased glucose-6-phosphatase, decreased glucokinase, impaired glucose tolerance and disrupted the structural integrity of the pancreas. Treatment with saxagliptin, metformin and their combinations significantly decreased blood glucose level, decreased LDL Level, improved glucose tolerance and offered protection to the pancreatic islet cells. In conclusion, the selected hypoglycemic agents used in present study ameliorate the dexamethasone induced hyperglycemia and insulin resistance of which the combination of metformin with saxagliptin showed greater efficacy.

Keywords*: Insulin resistance, Hyperglycemia, Dexamethasone, Type 2 Diabetes*

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INTRODUCTION

The burden of type 2 diabetes is a global health concern and the cost of managing this condition is worrisome especially to low- and middle-income countries. According to the International Diabetes Federation (IDF) estimation, about 415 million people worldwide were affected with Type 2 diabetes as of 2015, a number which is still on a rise and expected to reach around 642 million by 2040 (Cersosimo *et al*., 2018).

Type 2 diabetes is usually known to develop more at adult age, affecting mainly the elderly and/or obese individuals. Unfortunately, this disease is increasingly prevalent in young adults. This is particularly in highly susceptible ethnic groups due to the increase in childhood obesity (Petrovick 2018). Some of the commonly known risk factors of type 2 diabetes include being overweight and obesity (particularly of the android type), age, inactivity, family history, etc. Asides these, several drugs have been linked with an increased risk development of type 2 diabetes, among which includes Glucocorticoids (Pasieka & Rafacho 2016).

Glucocorticoids are among the most prescribed drugs worldwide for their therapeutic benefits in the treatment of several inflammatory and immunological diseases. Despite their therapeutic potentials in ameliorating these diseases, they have the drawback of inducing insulin resistance in humans. This can ultimately lead to steroid-induced diabetes or worsen previously diagnosed diabetes (Martinez *et al*., 2016). The diagnosis and treatment of glucocorticoid induced diabetes mellitus are surprisingly underestimated by many health practitioners and the media disclosure of the benefit with the use of dexamethasone in patients with COVID-19 infection sets the habits for self-medication and inappropriate use of glucocorticoids for some individuals

(Alessi *et al*., 2020). There are several drugs or combination of drugs with differing mechanisms of action that are used for treatment of type 2 diabetes mellitus (Davidson 2002). But despite advances in both our understanding of the pathophysiology of type 2 diabetes mellitus and the development of new treatment strategies, current management of patients with T2DM still remains sub-optimal (Lavernia *et al*., 2015). Furthermore, there are limited studies that compare the effectiveness of different treatment therapies as a means of assessing the effectiveness of preventive measures taken against glucocorticoids induced diabetes mellitus.

This present study is designed to evaluate the effect(s) of saxagliptin, metformin and intranasal insulin used singly and in combination in glucocorticoids -induced insulin resistance.

MATERIALS AND METHODS

Drugs and Chemicals: Recombinant human insulin (Actrapid, 100 IU/ml, Novo Nordisk) and Dexamethasone was purchased from Rotamedix Pharmacy Ilorin, Nigeria. Metformin (Glucophage) and Saxagliptin (Onglyza) was purchased from Momrata Pharmacy Ilorin, Nigeria. Isoflurane was obtained from Southern Anaesthesia & Surgical, (West Columbia).

Animals: housed in groups of seven and acclimatized for one week in the housing facility of the College of Health Sciences, University of Ilorin, Nigeria. Animals were housed under standard environmental conditions at a temperature of about 25o C. Animals had free access to water and formulated rat's chow ad libitum. All procedures involving animals were approved prior to the experimental phase. Ethical approval was obtained from the University Ethical Review Committee (UERC/ASN/2020/2032) and in compliance with the Helsinki declaration on the care and use of laboratory animals (World Medical Association 2020).

Experimental design: Fifty-six female wistar rats weighing between 180-230 grams were randomly distributed into eight groups (n=7). Dexamethasone was administered for a period of 7 days an hour before the administration of metformin, saxagliptin and intranasal insulin.

Group 1 - Control group received normal saline (0.9%)
Group 2 - Untreated group received intraperitoneal Dexamethasone injection (1mg/kg/day).
Group 3 was administered dexamethasone(1mg/kg) and were treated with intranasal insulin (2IU/day)
Group 4 was administered dexamethasone(1mg/kg/day) and were treated with the combination of intranasal insulin and orally administered Metformin (40mg/kg/day).
Group 5was administered dexamethasone(1mg/kg/day) and were treated with the combination of intranasal insulin and orally administered Saxagliptin (8mg/kg/day)
Group 6was administered dexamethasone(1mg/kg/day) and were treated with oral administration of metformin (40mg/kg/day).
Group 7 was administered dexamethasone(1mg/kg/day) and were treated with oral administration of metformin (8mg/kg/day).
Group 8 was administered dexamethasone(1mg/kg/day) and were treated with the combination of orally administered metformin (40mg/kg/day) and saxagliptin (8mg/kg/day).

Induction of Insulin Resistance: Induction of insulin resistance was done using the method employed by Martinez *et al*. (2016). Insulin resistance was induced by intraperitoneal injection of dexamethasone (1 mg/kg) for 7 days before the administration of saxaglipitin, metformin and intranasal insulin.

Intranasal Insulin Administration Procedure: Intranasal insulin administration was done in line with the method employed by Njan *et al*., (2018). Rats were anesthetized by placing them in tightly sealed transparent glass jars containing isoflurane (5%) for brief period (< 30 s). A total of 2 IU (1 IU/ 10 µl; 10 µl/nostril) of rapid acting insulin was quickly administered intranasally using a micropipette (P-10, Eppendorf). The procedure lasted for about 10 to 15 seconds. Animals that regain consciousness was monitored and returned to their respective cages.

Oral Glucose Tolerance Test: The Oral Glucose Tolerance Test (OGTT) was performed on overnight fasted rats and after which basal glycemia measurement on the 6th day. Each animal received orally, 2 g/kg of glucose and their glycemia was further measured at 30, 60, 90 and 120 min after glucose load (Chaimum-aom *et al*., 2017). The blood glucose from the animals were measured using blood glucose test strips and glucometer (Accuchek).

Fasting Blood Glucose Measurement: Fasting blood glucose levels were measured with an ACCU check glucometer on the 8th day for all groups using samples collected from the tail vein after an overnight fast (12 hours).

Tissue collection: At the end of the experiments (on the 8th day), rats from each group were subjected to general anaesthesia by ketamine. Following decapitation, blood was collected in plain tubes. The rats were dissected, and the pancreas and liver were rapidly removed. Representative pancreatic fragments were taken and used for histological examination (Mohamed *et al*., 2014). Pancreas were removed and fixed in 10% buffered formalin and dehydrated by graded series of alcohol, embedded in paraffin, sectioned at 5 µm in thickness, and mounted on glass slides. Pancreatic sections were stained with Hematoxylin and Eosin (H & E) and assessed for tissue injury. Acinar damage was assessed based on the appearance of lining cells, its pyramidal structure, presence, or absence of inflammatory cells, and pyknotic nuclei. Damage to the islets of Langerhans was assessed based on the presence or absence of intra-islet hemorrhages, cellular infiltrates and nuclear pyknosis. The liver was removed and homogenized with 0.25M sucrose solution in the liver weight: volume ratio of 1:4 of the weight of the liver to that of the volume of the 0.25M sucrose solution and evaluated for the levels of glucokinase and glucose-6-phosphatase enzymes (Njan *et al*., 2018).

Biochemical Analysis: Serum high density lipoprotein (HDL)-cholesterol, triglycerides (TG), low density lipoprotein (LDL) and total cholesterol (TC) concentrations

were analyzed by enzymatic determination, using the kits purchased from Randox laboratories Ltd, United Kingdom. Serum insulin levels were analyzed by enzymatic determination using ELISA kit purchased from CalBiotech. Serum adiponectin levels were analyzed by enzymatic determination using rat adiponectin ELISA kit purchased from bioassay technology laboratory. Values were expressed in mg/dl. Serum glucagon levels were analyzed by enzymatic determination using rat glucagon ELISA kit purchased from bioassay technology laboratory. Values were expressed in ng/L.

Statistical Analysis: Data collected were cleaned and statistical analysis was performed with GraphPad Prism (version 8.0) statistical software using the one-way/ repeated ANOVA with Turkeys multiple comparison and Dunnet comparison test. Values of $p \leq 0.05$ were considered significant. Values are expressed as Mean ± SEM; *P-value ≤ 0.05, **P-value <0.002, ***P-value < 0.001.

RESULTS

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Body Weight: Repeated intraperitoneal administration of dexamethasone (1mg/kg) for period of 7 days significantly decreases the body weight (grams) of the animals when compared to control group #. Oral administration of metformin (40mg/kg), saxagliptin (8mg/kg) or intranasal insulin (2IU) and their combinations could not significantly reverse the weight loss caused by dexamethasone (Figure 1).

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Blood Glucose Level.: From the chart (Figure 2), intraperitoneal administration of dexamethasone (1mg/kg) increased the fasting glucose levels in comparison with the control group . The administration of metformin (40mg/kg) and saxagliptin (8mg/kg) alone significantly reduced the fasting glucose levels when compared with the dexamethasone group*. Also, the combination of metformin (40mg/kg) with saxagliptin (8mg/kg), and combination of intranasal insulin (2IU) with saxagliptin (8mg/kg) significantly reduced the fasting blood glucose levels when compared to the dexamethasone group*. Combination of metformin (40mg/kg) and saxagliptin (8mg/kg) was significantly lower compared to intranasal insulin alone group.

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combinations on Oral Glucose Tolerance Test and Area Under the Curve.: Figure (3a) shows the OGTT curve in control and experimental animals following oral administration of 2g/kg of 40% glucose solution. Glucose peaked in the normal group and returned to baseline within 120 minutes. Dexamethasone treatment caused elevated fasting blood glucose relative to the normal control. Further glucose impairment was observed as blood glucose level did not return to normal at 120 minutes. Treatment with intranasal insulin also impaired glucose tolerance as blood glucose level was higher than dexamethasone at 120 minutes. Metformin treatment reduced blood glucose to levels comparable to normal control whether administered alone or in combination with intranasal insulin. Also, saxagliptin treatment reduced blood glucose to levels comparable to normal control whether administered alone or in combination with intranasal. Combination of metformin and saxagliptin improved glucose tolerance as blood glucose peaked at 60 minutes and fell to base line at 120 minutes.

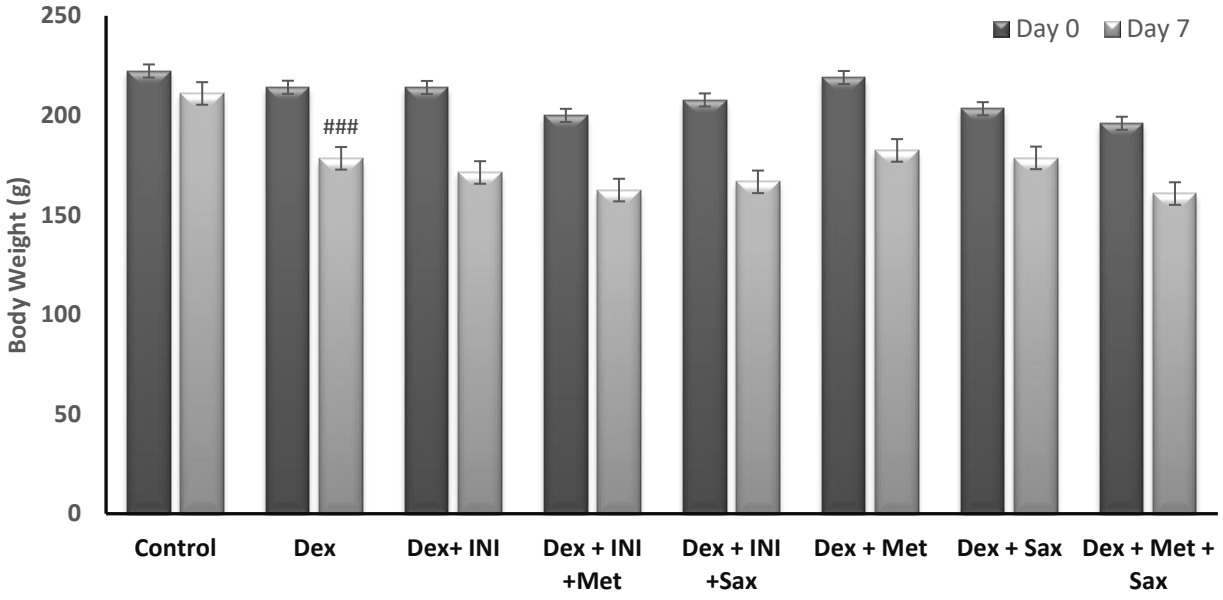

Figure 1.
Showing the effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on body weight measured at day 0 and day 7 of drug administration.

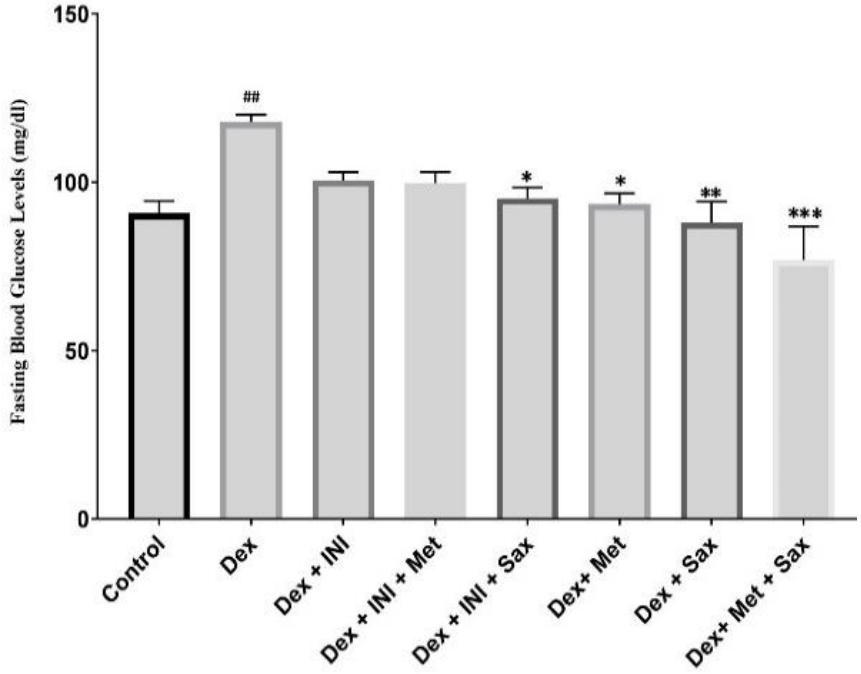

Figure 2:
Showing the effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Blood Glucose level.

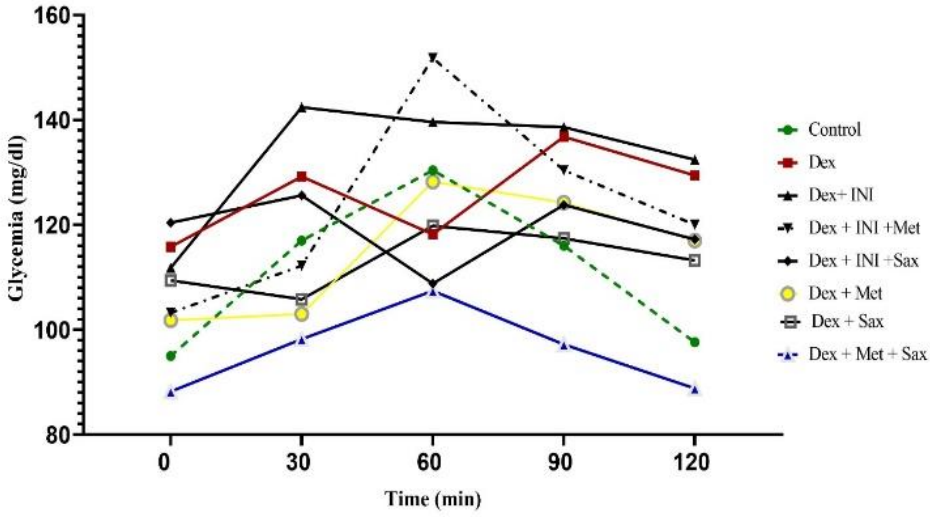

Figure 3(a):
Showing effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combinations on Oral Glucose Tolerance Test**.** Values are expressed in mean ± SEM (n=5).

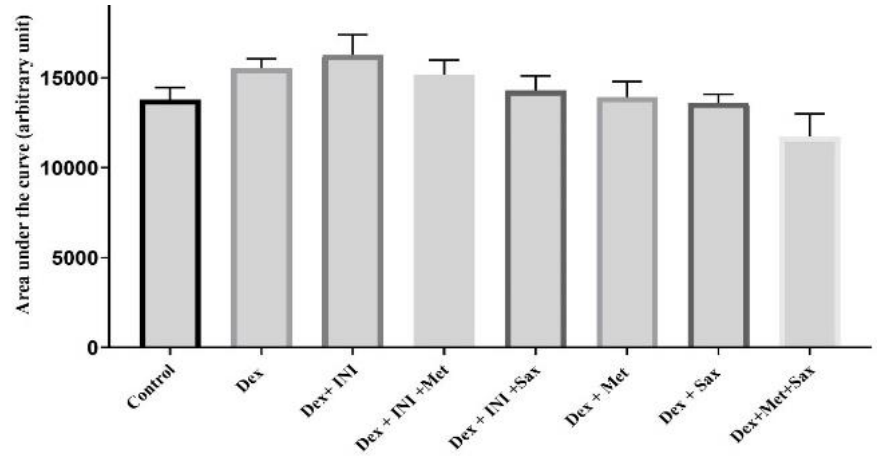

Figure 3 (b):
Showing the effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combinations on AUC from oral glucose tolerance test.

The figure (3b) shows the glucose Area under the Curve (AUC) in control and experimental animals treated with or without dexamethasone, intranasal insulin, metformin and saxagliptin. Dexamethasone treatment led to non-significant increase in AUC. The combination of metformin and saxagliptin however significantly reduced glucose AUC when compared with intranasal insulin alone group.

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Serum Insulin Level.: Intraperitoneal administration of dexamethasone (1mg/kg) resulted in non-significant increase in serum insulin levels in comparison with the control group (mean values 0.044 ±0.01 vs 0.032± 0.01). Oral administration of saxagliptin (8mg/kg) significantly increased serum insulin levels in comparison to dexamethasone alone and all other administered hypoglycemic drugs with p-values respectively (Figure 4).

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Fasting Serum Glucagon Level: From the chart below (Figure 5), intraperitoneal administration of dexamethasone (1mg/kg) increased serum glucagon levels in comparison with the control (mean values 287.50 ± 6.81 vs 213.91 ± 40.11). Except for combination of insulin with saxagliptin, other hypoglycemic agents administered reversed the dexamethasone induced hyperglucagonemia**.**

Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combinations on fasting serum Adiponectin level: The serum level of adiponectin was not significantly affected by dexamethasone (1mg/kg) administration (Figure 6). Although a slight decrease in adiponectin was observed when compared to the control group (mean values of 3.58 ± 0.17 vs 3.77 ± 0.11). All hypoglycemic agents administered were able to reverse the effect caused by dexamethasone. Combination of metformin (40mg/kg) with saxagliptin (8mg/kg) significantly increased adiponectin level in comparison to dexamethasone only group* intranasal insulin alone group, intranasal insulin + metformin intranasal + saxagliptin metformin alone group and saxagliptin alone group.

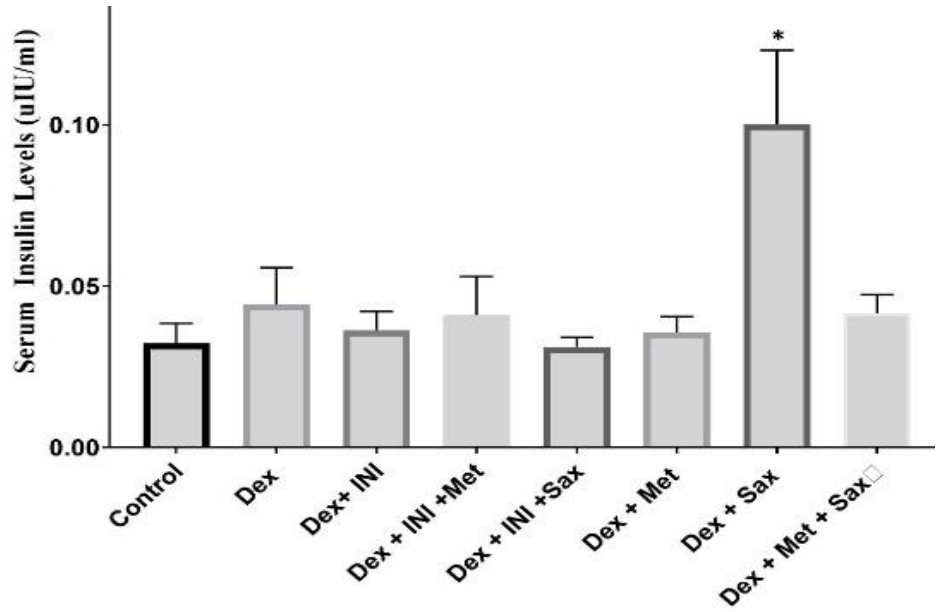

Figure 4.
Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on fasting serum insulin level

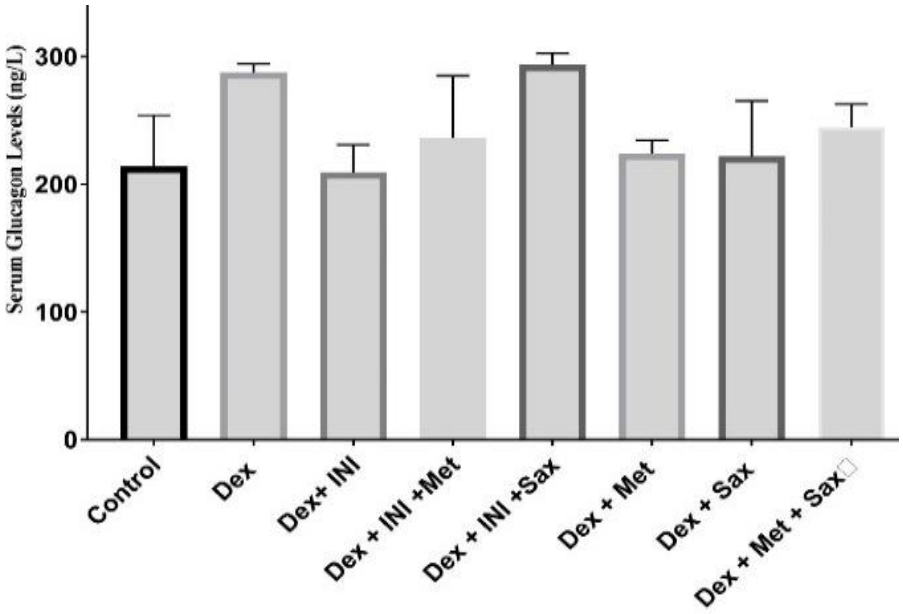

Figure 5.
Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on fasting serum glucagon level.

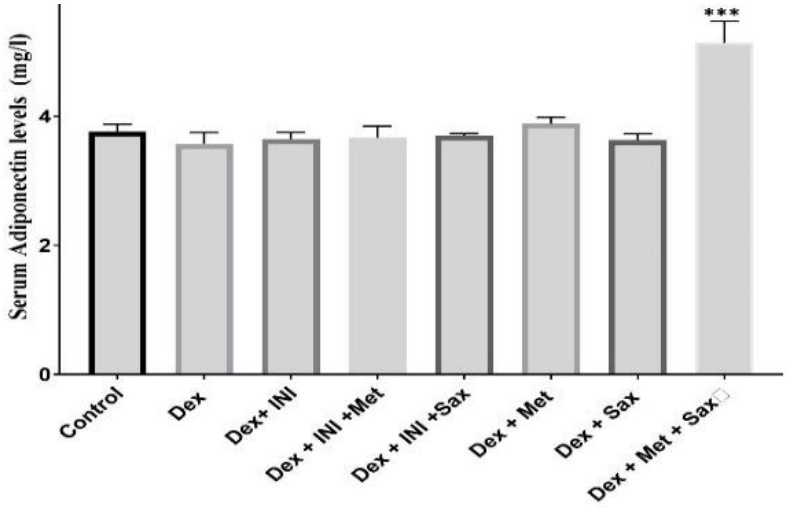

Figure 6:
Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on fasting serum Adiponectin level.

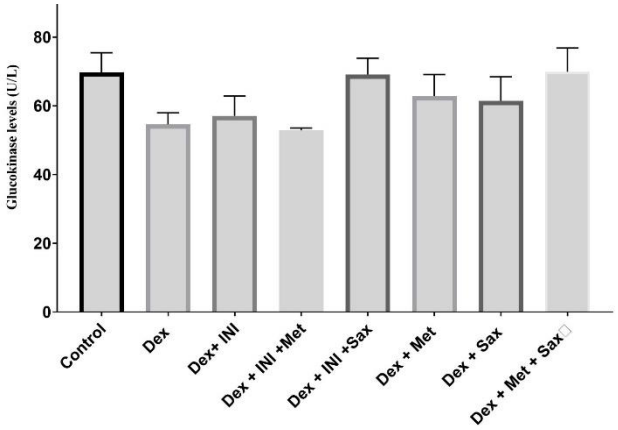

Figure 7a:
Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on glucokinase enzyme levels.

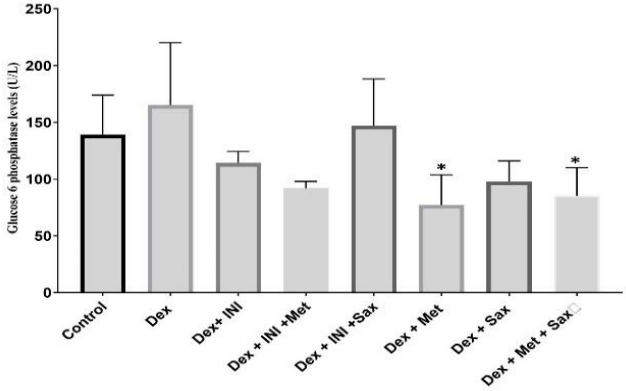

Figure 7b:
Showing the effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on glucose-6-phosphatase enzyme levels.

Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on liver enzymes: Intraperitoneal administration of dexamethasone decreased the level of glucokinase enzyme in comparison with the control (mean values 54.5787 ± 3.39 vs 69.7707 ± 5.64). All hypoglycemic agents increased the level of glucokinase enzyme in comparison to dexamethasone alone group, although none of the increase was significant (Figure 7a). Combination of insulin and saxagliptin was able to normalize the levels of the enzymes in comparison to control (mean values of 69.09 ± 6.99 vs 69.77 ±5.64). Also, intraperitoneal administration of dexamethasone (1mg/kg) increased the levels of glucose -6- phosphatase enzyme (figure 7b) in comparison with the control group (mean values 165.224 ± 27.50 vs 139.362 ± 34.55). All hypoglycemic agents decreased the levels of glucose -6-phosphatase in comparison to dexamethasone alone group. Among which the administration of metformin (singly) and the combination of metformin (40mg/kg) with saxagliptin (8mg/kg) caused significant lowering of the level glucose-6- phosphatase compared to dexamethasone group *.

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on Lipid Profile: Administration of dexamethasone significantly increased the serum total cholesterol level (Figure 8a) in comparison with the control #. The hypoglycemic agents reduced the level of total cholesterol compared to the dexamethasone group, among which the combination of intranasal insulin (2IU) with saxagliptin (8mg/kg) and combination of metformin (40mg/kg) with saxagliptin (8mg/kg) significantly reversed the increase in total cholesterol level induced by dexamethasone. Administration of dexamethasone significantly increased the serum Triglyceride levels (Figure 8b) in comparison to the control group. Combination of metformin (40mg/kg) with saxagliptin (8mg/kg) significantly decreased the serum triglyceride levels in comparison with the dexamethasone group # and saxagliptin alone group respectively. Administration of dexamethasone increased the serum HDL levels in comparison with the control (Figure 8c). The hypoglycemic agents employed in this study also increased the serum levels of HDL. Administration of dexamethasone (1mg/kg) significantly increased the serum LDL level (Figure 8d) in comparison with the control #. Except for the combination of metformin (40mg/kg) with intranasal insulin (2IU), all other hypoglycemic agent reversed the increased serum LDL caused by dexamethasone. The level to which the combination of intranasal insulin + saxagliptin and metformin + saxagliptin reversed this effect was significant compared to the group that received combination of intranasal insulin with metformin, saxagliptin alone and metformin alone group.

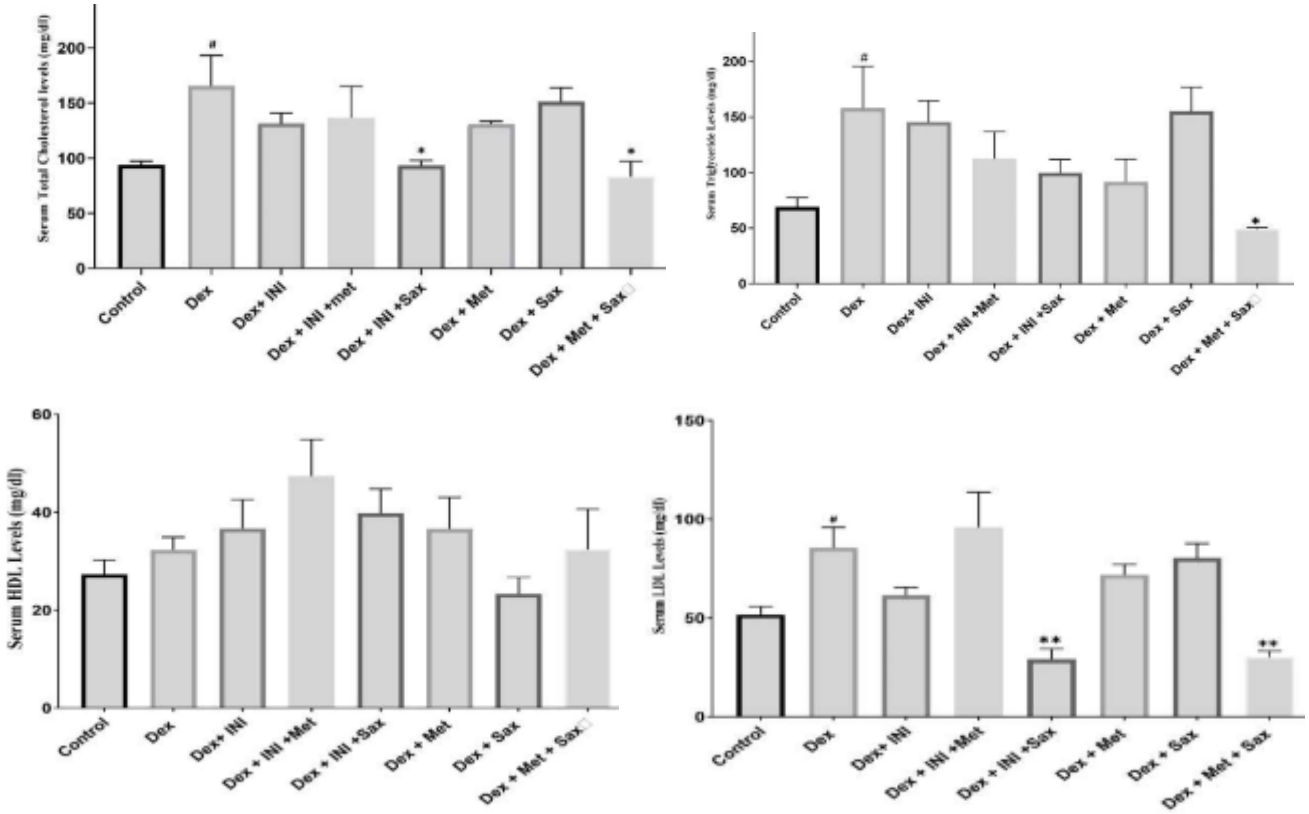

Figure 8:
a. Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum total cholesterol level.
b. Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum triglyceride level.
c. Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum HDL level.
d. Effect of dexamethasone, intranasal insulin, metformin, saxagliptin and their combination on serum LDL level.

Effect of Dexamethasone, Intranasal Insulin, Metformin, Saxagliptin and their combination on the Histology of the Pancreas: No visible lesion or damage was observed in the structure and architecture in the Hematoxylin and Eosin-stained sections of the pancreas of the control group (Figure 9a). Dexamethasone 1mg/kg showed pancreatic islet cells multifocal nuclear hypochromasia, cytoplasmic degeneration and individual nuclear loss necrosis (Figure 9b). Intranasal Insulin (2IU) administration showed pancreatic parenchyma acinar cell hyperchromasia with intact islet (Figure 9c). Treatment with combination of intranasal insulin (2IU) and Metformin showed mild to severe pancreatic parenchyma acinar cell hyperchromasia with intact pancreatic islet (Figure 9d). Combination of intranasal insulin (2IU) and Saxagliptin (8mg/kg) showed pancreatic acinar cell nuclear hyperchromasia and islet cell degeneration and necrosis with regeneration (Figure 9e). Metformin (40mg/kg) alone showed severe pancreatic parenchyma acinar cell hyperchromasia with intact pancreatic islet cells (Figure 9f). Saxagliptin (8mg/kg) alone showed pancreatic islet cell degeneration and necrosis (Figure 9g). Combination of metformin and saxagliptin showed moderate pancreatic parenchyma acinar cell nuclear hyperchromasia with intact acinar cells and islet cells (Figure 9h)

DISCUSSION

A key challenge in the excessive use of dexamethasone is its ability to promote adverse metabolic effects which include insulin resistance and can as such induce or worsen previously diagnosed diabetes (Pasieko & Rafacho 2016). The present study was carried out to compare the effect of selected hypoglycemic agents on dexamethasone induced insulin resistance. Dexamethasone confers a risk of weight gain in humans (Wang *et al*., 2012). However, in this study, repeated administration of dexamethasone caused a significant reduction in body weight of animals. In support of the observed weight loss, Malkawi *et al*, (2018), suggested that the mechanism of dexamethasone effect could be due to muscle wasting.

Alterations in glucose homeostasis, insulin resistance and hyperglycemia are amongst the adverse effects that have been tied to glucocorticoids therapy (Pasieka and Rafacho 2016). In line with this, present study showed that dexamethasone significantly increased fasting blood glucose levels compared to control. Except for the administration of intranasal insulin alone and its combination with metformin, other hypoglycemic agents showed a significant reduction in blood glucose. Metformin has been the most preferred first line agent employed in the management of Type 2 diabetes mellitus (Pretrovick 2018), but the current study indicated that saxagliptin administered may be superior to metformin and intranasal insulin at lowering fasting blood glucose. The combination of metformin and saxagliptin offered better therapeutic benefits compared to the other combination/single regimen used in this study.

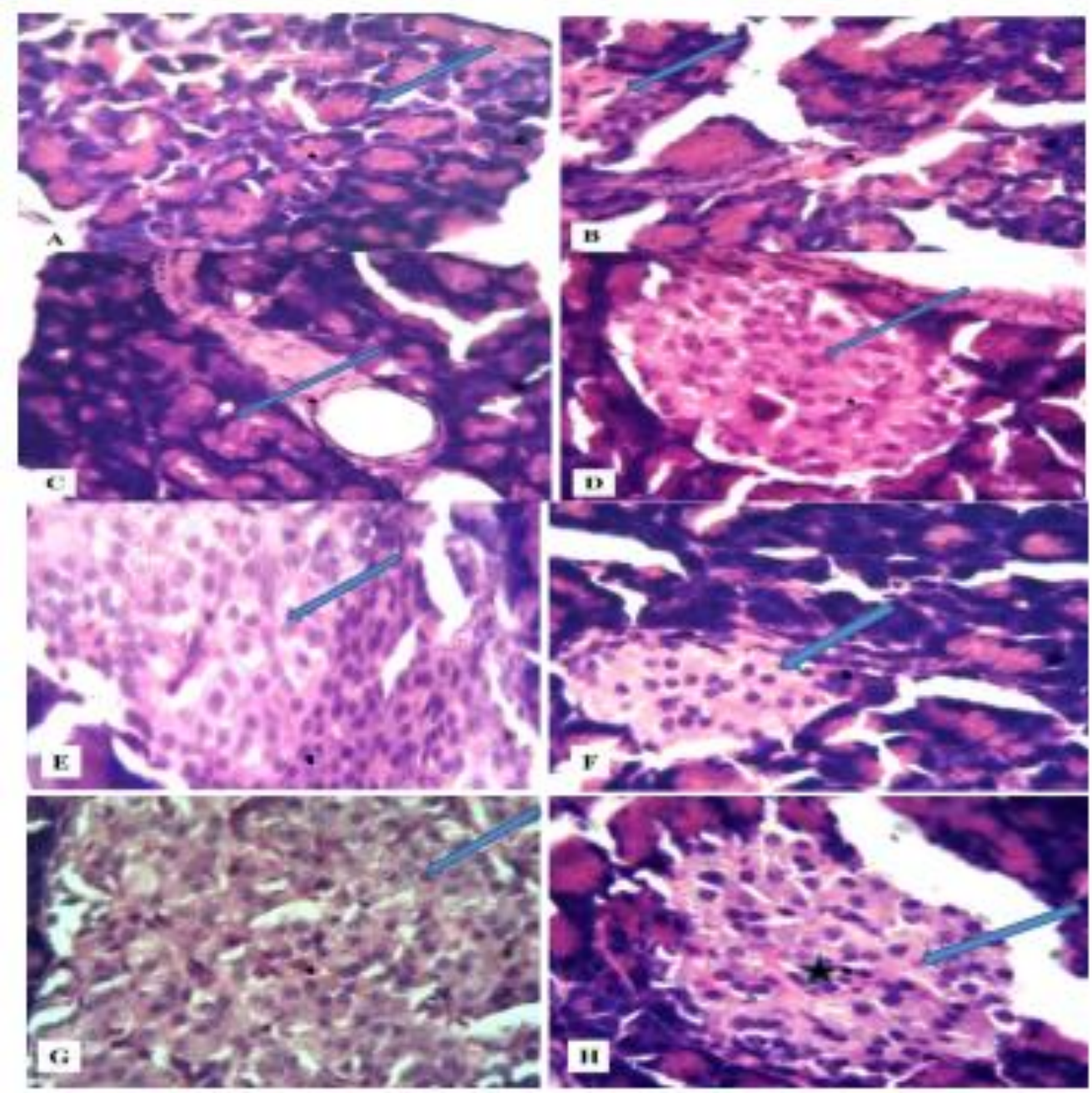

Figure 9.
H & E-stained microscopic photomicrograph of the different studied groups in Magnification x400. Arrows indicating the pancreatic islet cells.

To assess insulin sensitivity, oral glucose tolerance test done in present study indicated an impaired glucose tolerance in the dexamethasone group as blood glucose level peaked within 30 to 90 minutes following glucose load and did not return to baseline after a period of 2 hours. The mechanisms by which glucocorticoids impair glucose tolerance are not yet fully clarified in humans and merit continuous research. However, metformin, saxagliptin and their combination improved glucose tolerance compared to the dexamethasone group. This result reflects the ability of metformin and saxagliptin to ameliorate insulin resistance when used alone and in synergy when combined (Lindsay *et al.*, 2005). Intranasal insulin administered as monotherapy did not improve glucose tolerance, which is consistent with an early study (Njan *et al.*, 2018) and reflects the inability of intranasal insulin to improve insulin resistance within a short term of treatment. However, combining intranasal insulin with metformin or saxagliptin improved glucose tolerance which could possibly due the additive effects of metformin or saxagliptin on glucose homeostasis when combined with intranasal insulin. Furthermore, daily administration of dexamethasone increased the activity of Glucose-6-phosphatase (Gluconeogenic enzyme) which is known to evoke gluconeogenesis (Jitrapakdee 2012). Intranasal insulin, metformin, saxagliptin and their combinations was able to reduce the activity of Glucose-6-phosphatase enzyme of which metformin alone and its combination with saxagliptin proved to be significant. This may be one of the possible mechanisms to justify the combination of metformin with saxagliptin at preventing dexamethasone-induced hyperglycemia and impaired glucose tolerance.

Dexamethasone -induced increase in total cholesterol level, triglyceride level and LDL observed in present study is line with those reported by Arab and Mahboubi (2015) except that, dexamethasone administered in this study increased HDL concentration. The mechanism of dexamethasone induced increase in HDL is still not entirely clear, but this is consistent with previous study by Wang *et al*., 2012. Intranasal insulin, metformin, saxaglitin and their combinations used in present study was able to reduce Total cholesterol levels, Triglyceride levels, LDL and increase HDL of the combination of saxagliptin with intranasal insulin and its combination with metformin proved to be more efficacious. This result gives an insight of the potentials of these hypoglycemic drugs at reducing or preventing cardiovascular related diseases such as retinopathy, nephropathy, stroke, and coronary artery disease (CAD).

Several prospective studies have linked low levels of adiponectin with insulin resistance, metabolic syndromes and as a contributing factor to the progression from a prediabetes state to frank type 2 diabetes mellitus (Duncan *et al*., 2004; Han *et al*., 2009). In this regard, dexamethasone administered in present study had no significant modifiable effect on adiponectin levels and neither of intranasal insulin, saxaglitin or metformin was able to increase the levels of adiponectin within the short period of administration. Interestingly, combination of metformin with saxagliptin significantly increased adiponectin levels in comparison to all other groups. Glucokinase enzyme which has a very powerful control on glucose disposal by promoting the storage of glucose in liver cells as glycogen (Wilamowitz *et al*., 2013) was explored in present study to further justify the effect of selected hypoglycemic agents at ameliorating dexamethasone induced hyperglycemia. Dexamethasone reduced the activity of this enzyme amongst which the combination of saxagliptin with intranasal insulin and the combination of saxagliptin with metformin was able to normalize this effect. In line with a previous study (Narendar *et al*., 2015), administration of dexamethasone in present study increases serum insulin levels suggesting enhancement of beta-cell function to compensate for the increased glucocorticoid-induced peripheral insulin demand. Saxagliptin alone also significantly increased the level of insulin compared to all other hypoglycemic agents employed in present as an evident of its mechanism of action (Li *et al*., 2016). To further support the concept of dexamethasone-induced hyperglycemia (Sofie *et al*., 2018), result from this study also indicated an increase in glucagon activity. Except for the combination of saxagliptin with intranasal insulin, all other hypoglycemic agents explored in present study were able to reduce the level of glucagon relatively closer to the control group. Correlating with the biochemical assays conducted in this study, hyperglycemia and hyperlipidemia have been showed to fuel alterations in certain biochemical pathways, such as oxidative stress, low-grade inflammation, and apoptosis which could precipitate the development of insulin resistance and beta-cell dysfunction (Tangvarasittichai 2015). Results from histological investigation of the pancreas using H&E stain, indicated that our short-term administration of dexamethasone caused an atrophy of pancreatic islet cells. The goal of hypoglycaemic therapy is to achieve a tight glycaemic control and further prevents cardiovascular complications with possible preservation of beta-cell function. Except for saxagliptin alone group of which sectioned part showed degeneration of the islet cell, all other hypoglycaemic agents employed in present study offered protection to the pancreatic islet cells. Previous reports from three cross-sectional studies (Scirica *et al*., 2013, Chein-feng lee *et al*., 2014, Raz *et al*., 2014,) have linked the extended use of saxagliptin to be associated with small but significant recurrent acute pancreatitis despite its efficacy and long clinical success. This present study establishes the effect of dexamethasone on hyperglycemia induction and impaired glucose tolerance and the role of selected hypoglycemic agents in ameliorating these effects and of which addition of saxagliptin to metformin therapy proved to be most effective.

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Full length Research Article

Neurobehavioural and Histological Study of the Effects of Low-Dose and High-Dose Vanadium in Brain, Liver and Kidney of Mice

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Summary: Vanadium is a ubiquitous transition metal that has been generating contrasting research interest. Therapeutically, vanadium possess antidiabetic, antitumor, antiparasitic and even neuroprotective activities. On the flip side, vanadium has been reported to cause multisystemic toxicities with a strong predilection for the nervous system. Despite several reports on potential benefits of low-dose vanadium (LDV) and toxic effects of high-dose vanadium (HDV), there are no comparative studies done thus far. This study therefore explored the comparative effects of LDV and HDV exposure in mice during postnatal development. A total of nine (9) nursing mice were used in this study; with three nursing mice and their pups (n = 12 pups per group) randomly assigned to each of the three test groups. The nursing dam were given intraperitoneal (i.p) injection of vanadium at 0.15mg/kg and 3mg/kg for LDV and HDV respectively, and subseqently to the pups from postnatal day (PND) 15 till sacrifice on PND 90. We discovered that neurodevelopmental motor function test of mice-pups exposed to LDV here showed improved motor development, muscular strength and memory capacities whereas HDV led to motor function impairment, reduced muscular strength and memory capacities. LDV-exposed mice showed mild histological lesions in cerebral cortex whereas high-dose showed distinct histological lesions in different parts of the brain ranging from cerebellar Purkinje neuronal pathology (central chromatolysis), pyramidal neuronal loss in CA1 region, architectural distortion as well as fewer neurons in olfactory bulb. We saw mild lesions with LDV in both liver and kidney, however, with HDV exposure, there was diffuse hepatocellular vacuolar degeneration and congestion of blood vessels in liver, shrinkage of renal glomerulus and degenerated epithelial cells of kidney. Conclusively, beneficial effect of vanadium is proven as it facilitated body weight gain which translate in organ weight at low-dose, while high-dose caused decreased neurobehaviour and histological lesions.

Keywords*: Histological study, Neurobehavioral tests, High-dose vanadium, Low-dose vanadium*

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INTRODUCTION

Vanadium is classed as a transition metal that is present in nearby environmental sources like water, soil, air and also in the biological system of living organism (Pessoa *et al*., 2015). In the commercial world, vanadium is used in manufacturing and processing of pesticides, sulphuric acid, hardening of steel and as a catalyst in the production of many materials (Fatola *et al*., 2019). Exposure of vanadium happens from various sources such as by-products of burning fuel-oils laden with vanadium (Amorim *et al*, 2007) and mining of heavy metals (Moskalyk and Alfantazi, 2003). Small amounts of vanadium are beneficial to the growth and development of animals and its deficiency in mammals inhibits growth, impairs the generative functions, thyroid metabolism and bone mineralization, and disturbs the lipid and carbohydrate balance (Nilsen and Uthus, 1990 and Facchini *et al*., 2006). Therefore, vanadium is a necessary ingredient of the daily diet (French and Jones 1993; Rojas *et al*., 1999; Moskalyk and Alfantazi 2003). The following properties: antibacterial, anti-carcinogenic, spermicidal, anti-viral, anti-parasitic, anti-HIV, antituberculosis, anti-atherosclerotic, anti-hypertensive, as well as anti-thrombotic have been further documented as uses of vanadium compounds (Omayone *et al*., 2020). However, the major focus in their use is for anti-diabetic drugs because they are known to exhibit hypoglycaemic property (Huang *et al*., 2014; Novotny and Kombian, 2014; León *et al*., 2014; Rozzo *et al*., 2017; Jaiswal and Kale, 2019; Treviño *et al*., 2019).

On the other hand, exposure to larger dose of vanadium poses toxic risk to health as there have been reports of the toxicity in both humans (Rehder, 2013) and animal models (Olopade *et al*., 2011, Folarin *et al*., 2017, Igado *et al*., 2020). Higher dose of vanadium causes irritation of the eyes and mucous membranes of the upper respiratory duct, coughing, fatigue and depression (Goc, 2006). Also, neurobehavioral deficits such as impaired short-term memory, reduced reaction speed and loss of coordination were reported in workers exposed to vanadium in a steel factory in China (Li *et al*., 2013). In animal studies, vanadium has been shown to have toxic effects on various organ systems including the nervous, reproductive, gastrointestinal and urinary systems as reviewed (Wilk *et al*., 2017). Exposure period, concentration and means of administration influence the outcome of vanadium exposure. To date, there is scant information on the neurobehavioral effects and histological study of low-dose exposure to vanadium. This study aimed to compare low-dose (therapeutic dose) and high-dose (toxic dose) vanadium oral ingestion effects on neurobehaviour, body and brain weights and assess effects on cellular architectures of the brain, liver and kidney in the mice after exposure to both high-dose and low-dose vanadium for 90 days (PND 1 – PND 90).

MATERIALS AND METHODS

Animals and Treatments: Pups from nine (9) nursing mice were treated with vanadium for three months (PND 90). They were separated into three groups. We secured all animals from the Animal House, Department of Veterinary Anatomy, University of Ibadan, Nigeria. Vanadium as sodium metavanadate ($Na.O_3V$) was a product of Santa Cruz Biotechnology, Inc., Dallas. Schematic of the experimental setups is represented in Figure 1.

Control: The nursing dams were given daily intraperitoneal (i.p) injection of sterile water for duration of two weeks (days 14). The pups at PND 15 started receiving sterile water daily up to PND 30. Afterwards, the pups received i.p injection of sterile water every 72 hours (2 days interval) till sacrifice on PND 90.

Low-dose: The nursing dams were given daily intraperitoneal (i.p) injection of vanadium 0.15mg/kg for duration of two weeks (14 days). The pups at PND 15 started receiving vanadium daily up to PND 30. Subsequently, the pups received intraperitoneal (i.p.) injection of vanadium every 72 hours until sacrifice on PND 90.

High-dose: The nursing dam were given daily intraperitoneal (i.p.) injection of vanadium 3 mg/kg for duration of two weeks (14 days). The pups at PND 15 started receiving vanadium daily up to PND 30. Afterwards, the pups received i.p injection of vanadium every 72 hours until sacrifice on PND 90.

Body and Organ Weight: Every day we documented each pup's body weight from PND 1¬ – PND 30 and thereafter every 72 hours until they reached PND 90. The relative brain weights of all the three groups during sacrifice at PND 90 were harvested and weighed immediately, and measured based on the method described by Bailey *et al*., (2004) and Igado *et al*., (2020). The relative brain weights were later calculated as given below and then expressed in percentage.

$$\frac{\text{Brain Weight}}{\text{Body Weight}} \times 100$$

Cliff Aversion Test: Assessment of locomotor prowess as well as body strength of PND 2 and PND 7 mice was carried out using cliff aversion examination (Feather-Schussler and Ferguson, 2016). The mice pups were placed at the brim of a box without the nostrils or forearms touching but using just the elbows to hang. The time it takes each pup to face away from the box brim was documented. This assessment is repeated should a pup slip off the brim but interrupted if a pup does nothing in 30 seconds. The interruption was necessary to prevent the pups from getting accustomed to the test so as not to hamper analysis and the affected pup said to have failed the assessment.

Negative Geotaxis Test: Mice pups were evaluated at PND 2 and PND 7 using a plane, 450 inclined to the surface. We set the pups to face downward on the plane. After holding for five seconds, the time taken by the pup to face up the inclination (full 1800 turn) was recorded. 30 seconds maximum period was allowed. This latency to turn is a natural reaction by the pup against gravitational pull.

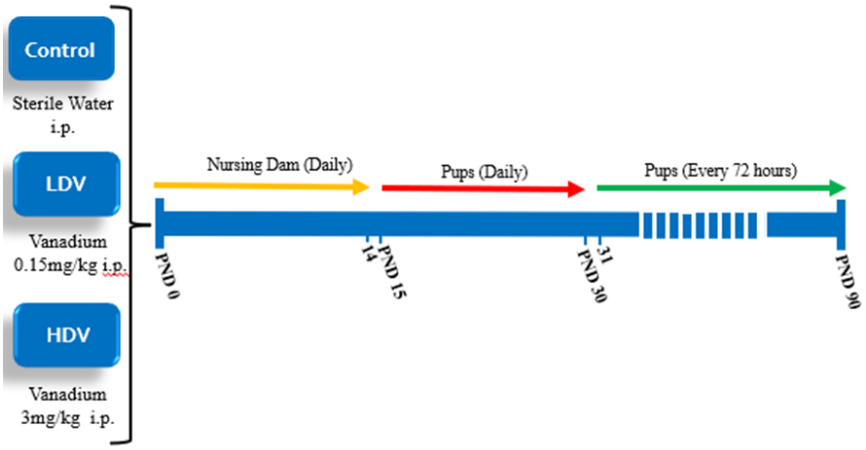

Figure 1:
Experimental setups

Forelimb Grasping Test: Evaluation of muscle strength and endurance involved subjecting the pups to forelimb grasping test at PND 14 and PND 21. A one-millimeter (1 mm) diameter wire was suspended horizontally while the pups were made to grip it with their forelimbs. The wire was positioned 50 cm above a soft-landing surface, and the time until losing grasp of the wire (Latency to drop) was noted (in seconds); a maximum cut-off time of 120 seconds was set.

Morris Water Maze: The Morris water maze is an evaluation tool designed to assess spatial learning and memory (Folarin *et al*., 2016). The test set up is a black circular tank measuring 110cm in diameter and 30cm in height. The tank was topped with water up to the 30cm level and we maintained a room temperature of 26°C. A hidden platform measuring 10 cm in diameter was utilized as the escape target. Eagerness of mice getting away from a water-lodged area using the shortest course forms the basis of this test. The tank tagged South (S), West (W), North (N) and East (E) had a platform concealed at a definite location. The period taken by a mouse, when placed in the tank of water, to locate such concealed platform was noted. Inability to discover platform in a 60-second period resulted in such mouse being led to locate and linger fifteen seconds on the platform. Every animal went through a first phase of training trial as well as a second phase of test (probe) in the Morris water maze test. The mice capability in learning the concealed platform's spatial location was assessed in the first phase for 3days in succession. Concealed platform being taken away in the second phase (on day 4), the period taken by the mice to remain within that quadrant of the platform was noted in evaluating the spatial memory.

Hanging Wire Test: A measure for forearm strength is done with the test. 2 beams set vertically carrying a pole placed at a 60cm-distance from a padded floor comprise the set up. We made PND 90 mice to grip the pole at the centre with the forearms. The time (in seconds) it took the mice to drop off the pole was documented. Failure to drop off after 120 seconds would result in the mice being released from the pole. Every mouse underwent this test twice with the mean time documented for eventual assessment.

Organ Sample Collection: Sacrifice of 5 mice in each group by post-natal day 90, was done after behavioural assessment. Every mouse was euthanized with ketamine (100mg/kg b.w.) and perfused transcardially using 10% Neutral buffered formalin (NBF) and brains harvested according to Olopade *et al*., (2011) method.

Tissue Processing for Microscopic Study: The brain samples went through paraffin-embedding routine process. 5µm-thick sections were produced using a microtome (Microm GmbH. D-6900 Heidelberg, West Germany) and stained with Haematoxylin and Eosin stain to evaluate general histology (Gilbert *et al*., 2020). Cresyl violet was used to stain the Nissl granules in the brain according to Folarin *et al*., (2017). Every stained slide was visualized using microscope (Leica Microsystems, Wetzlar, Germany).

Statistical analysis: We fixed the significance level of this study to 95%. and presented data as means ± SEM. Gradual alterations within various groups were assessed using one-way/two-way analysis of variance. At p-value<0.05, group variations were regarded significant. Every evaluation was carried out with GraphPad Prism (GraphPad Software, San Diego, version 5.0).

RESULTS

Body and Organ Weights: The effect of low-dose vanadium as compared to high-dose vanadium in mice exposed from postnatal day (PND) one for ninety days was assessed. At sacrifice (PND 90), the body weight of the mice exposed to low-dose was significantly higher than that of controls. Evaluation of relative body weight gain showed no statistical differences during the initial two weeks of postnatal development (from PND 1 – PND 13) across all the three groups. Significant differences in body weight occurred subsequently after two weeks both between LDV and HDV groups and between LDV and control groups (Figure 2).

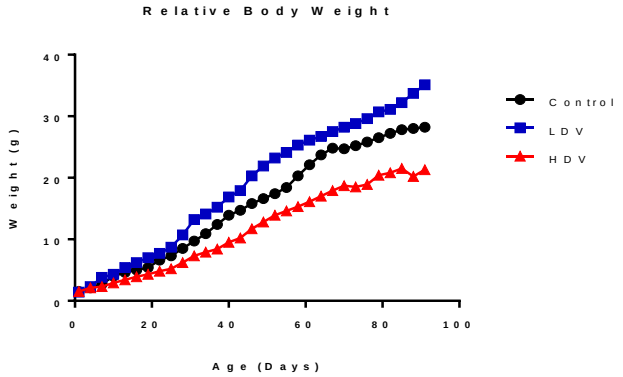

Figure 2:
Line graph showing the average body weight measurements of the mice pups from PND 1 – PND 90 for Control, LDV and HDV groups (n = 12 mice per group). *p<0.05, **p<0.01 statistically significant.

We observed across groups the same similarity in the weight of brain upon sacrifice of PND 90 mice. In all the three groups, the brain of the LDV group had a slightly higher weight than control but they are not statistically different. Both LDV and control groups had brain weight significantly higher than HDV group. But there was no statistical difference between LDV and control groups even though LDV had brain weight slightly higher than control (Figure 3).

Hanging Wire Test: The assessment of neurobehavior revealed alteration in locomotor ability in high-dose treated group relative to low-dose exposed group and control at both developmental stage and subsequently at adult stage. In the hanging wire test, the mice in the LDV group had improved muscle endurance while the HDV had decreased muscle strength leading to significantly shortened latency to fall at 3 months of age (PND 90). Low-dose vanadium group performed better than control but it was not statistically significant. Both control and low-dose vanadium treated

group had a statistically significant increase in performance when compared to high-dose exposed group (Figure 4).

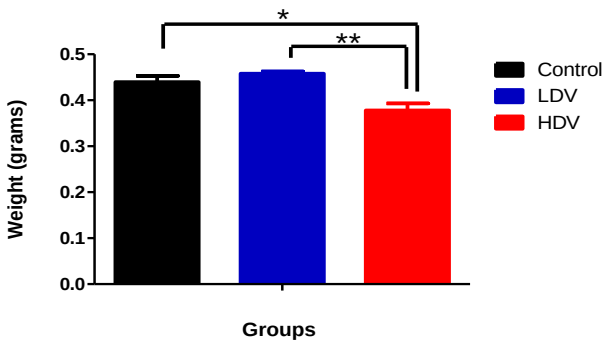

Figure 3: Bar chart graph showing relative brain weight. LDV had a relative brain weight that is higher than Control, however, not statistically significant. The relative brain weight of LDV was statistically significantly (**p< 0.01) higher than HDV. HDV group was higher than control group and it was statistically significant (*p< 0.05). Columns represent mean ± SEM.

Morris water Maze test: The Morris assessment test for all the three groups exhibit no statistical differences in learning capacities except for day 3 trial where low-dose vanadium and control groups had a significant learning ability than high-dose vanadium group (Figure 5A). Subsequently, there was a statistically significant memory impairment after exactly three months of high dose vanadium exposure when compared to control and low-dose vanadium treated groups. Furthermore, there was no statistical differences between control group and low-dose exposed group. However, low-dose treated mice shows slightly enhanced memory ability than control group (Figure 5B, 5C).

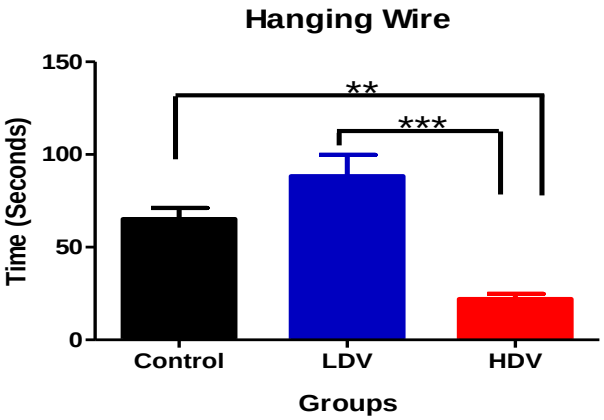

Figure 4:
Muscle strength assessment using wire hanging test among mice. Hanging time (s) was measured on PND 90. The low dose vanadium exposure improved muscle endurance in mice whereby high dose exposure decrease muscle strength. LDV performed better than control but it was not statistically significant. Both control and LDV had a statistically significant increase in performance when compared to HDV. Columns represent mean ± SEM.

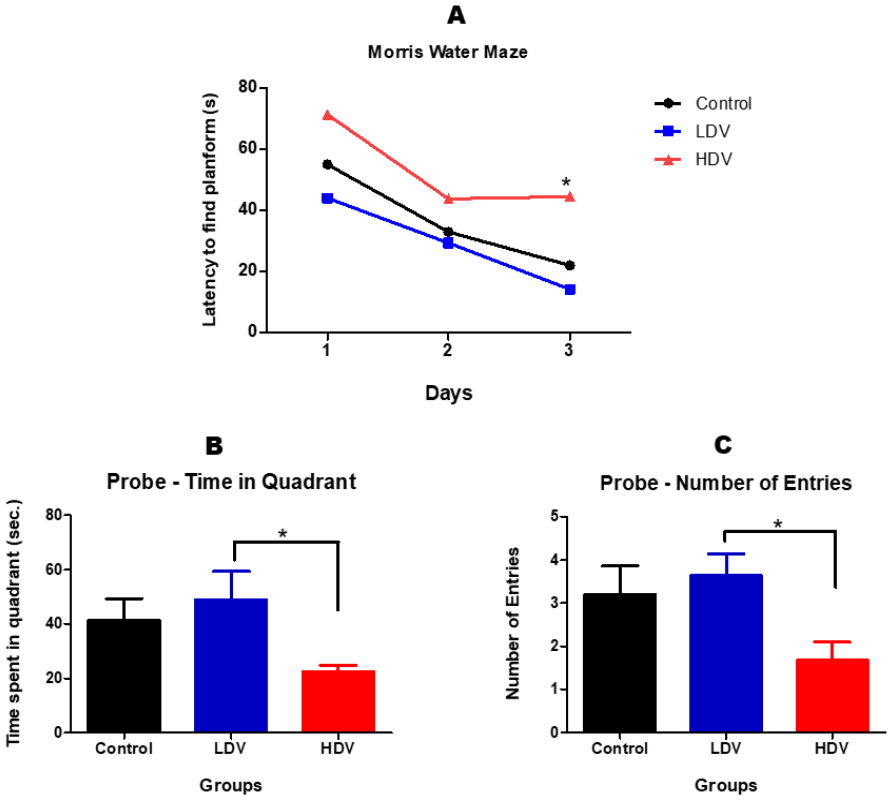

Figure 5:
Effect of low-dose and high-dose vanadium treatment for three months on learning and memory in mice. **A.** The ability to learn the location of the hidden platform was improved in all the groups as they all gradually spent shorter times to locate the hidden platform with subsequent trainings. However, there was a significant reduction in the rate of learning in the HDV-exposed mice compared to the LDV-exposed mice. **B.** During probe trial, the HDV-exposed mice spent a significantly shorter time in the target quadrant than the LDV-exposed mice ($*P <$ 0.05). Each point is the mean ± SEM. **C**. The number of entries of the LDV-exposed mice into the target quadrant was significantly higher than the HDV group ($*P < 0.05$). Each bar is the mean ± SEM.

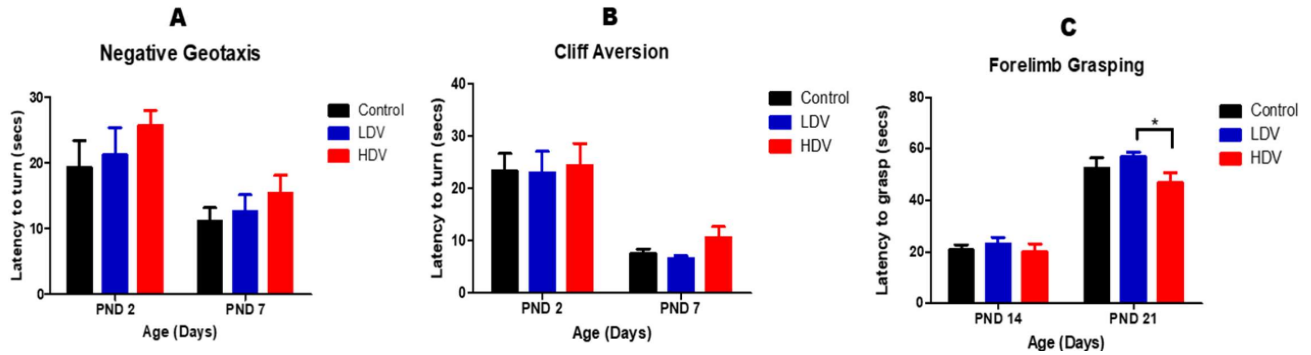

Figure 6:
Neurodevelopmental motor function test of mice pups exposed to low dose and high dose vanadium from PND 1. **A.** Exposure to LDV and HDV did not affect the latency to turn at PND 2. However, at PND 7, control group tended to perform better than LDV group but it is not statistically significant. **B.** LDV improved the ability of the mice to turn and avoid cliff than LDV group because they use shorter time in turning away **C.** LDV group exposure to low dose vanadium significantly improved latency to fall in the forelimb grasping test for pups at PND 21. Columns represent mean ± SEM. *P < 0.05.

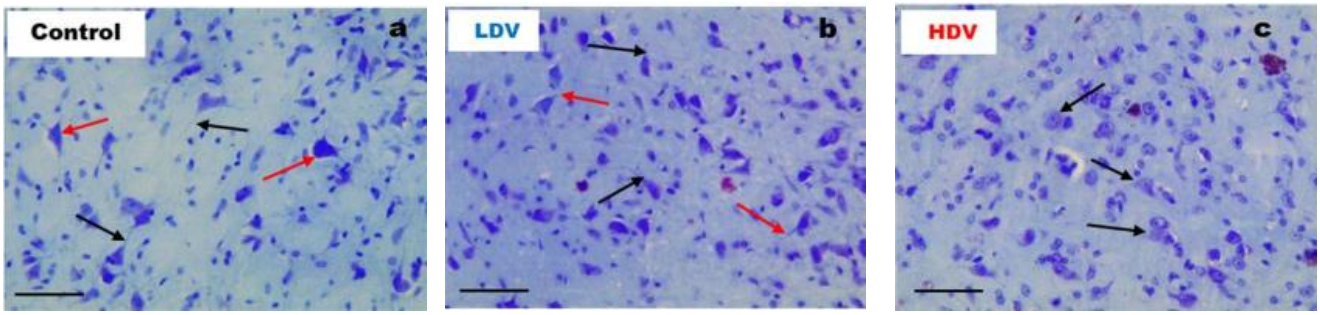

Plate 1:
Photomicrograph of cerebral cortex showing the pyramidal cell in mice. **a.** The control group showing normal pyramidal cells (red arrow) with dendritic extensions (black arrow). **b.** LDV group also showing relatively normal pyramidal cells (red arrow) that are still retaining some dendritic extensions (black arrow). **c.** The cerebral cortex of HDV showing multiple deformed pyramidal neurons (black arrow) lacking dendritic extensions. Cresyl Violet staining. x40 Magnification. Scale bar = 100 µm.

Developmental Test: Negative Geotaxis, Forelimb Grasping and Cliff Aversion: Neurodevelopmental motor function test of mice pups exposed to low-dose vanadium right from PND 1 showed improved motor development and muscular strength whereas high-dose vanadium led to motor function impairment and reduced muscular strength. In negative geotaxis test, the high-dose vanadium exposed group had a longer latency to turn away from gravitation when compared to control and low-dose vanadium treated groups which was statistically significant on both PND 2 and PND 7. However, there was no statistically significant difference between control and low-dose vanadium treated groups (Figure 6A). In the Cliff aversion test, high-dose vanadium exposed mice had significantly longer latency to turn with head and arm compared to control and low-dose vanadium exposed mice pups, while there was no significant difference between low-dose vanadium exposed mice and control (Figure 6B). The forelimb grasping abilities was significantly reduced for high-dose vanadium treated mice compared to the low-dose vanadium group. However, there was no significant difference between low-dose vanadium and control (Figure 6C)

Histological Examinations: The cerebral cortex in the control group had normal pyramidal cells with intact dendrites, the same was also seen in the low-dose vanadium exposed group. However, in the high-dose vanadium group, multiple deformed pyramidal neurons in the cerebral cortex which are lacking in dendritic extensions were observed (Plate 1).

The cerebellum in the control and low-dose vanadium groups had normal architectural arrangement of the molecular, granular and Purkinje cell layers. However, in the high-dose vanadium group, we observed degenerated Purkinje neuron that are pyknotic and are devoid of dendritic arborization. We also observed loss of Purkinje cells (Plate 2).

The pyramidal cells in the cornus ammonis 1 (CA 1) region were normal in shape and arrangement in the control and low dose vanadium groups. However, in the high-dose vanadium group, the cells appeared pyknotic, with distorted neuronal morphology and reduced layers of neuronal cells in this hippocampal region (Plate 3).

The control group showed normal cellular architecture of dentate gyrus. LDV group also showed normal dentate gyrus, however, there is a relative mild vacuolation of dentate gyrus. The dentate gyrus in the HDV presented multiple deformed neurons and severe vacuolation as compared to control and LDV groups.

The olfactory bulb in mice in the control and low-dose exposed vanadium had normal olfactory glomerular cells and normal architecture of mitral cells and granular cells. However, in the high-dose vanadium group, we observed some olfactory glomerular cells undergoing degeneration and reduced number of glomerular cells. Furthermore, the mitral cell layer exhibited distorted granular cells with fewer mitral cells (Plate 4).

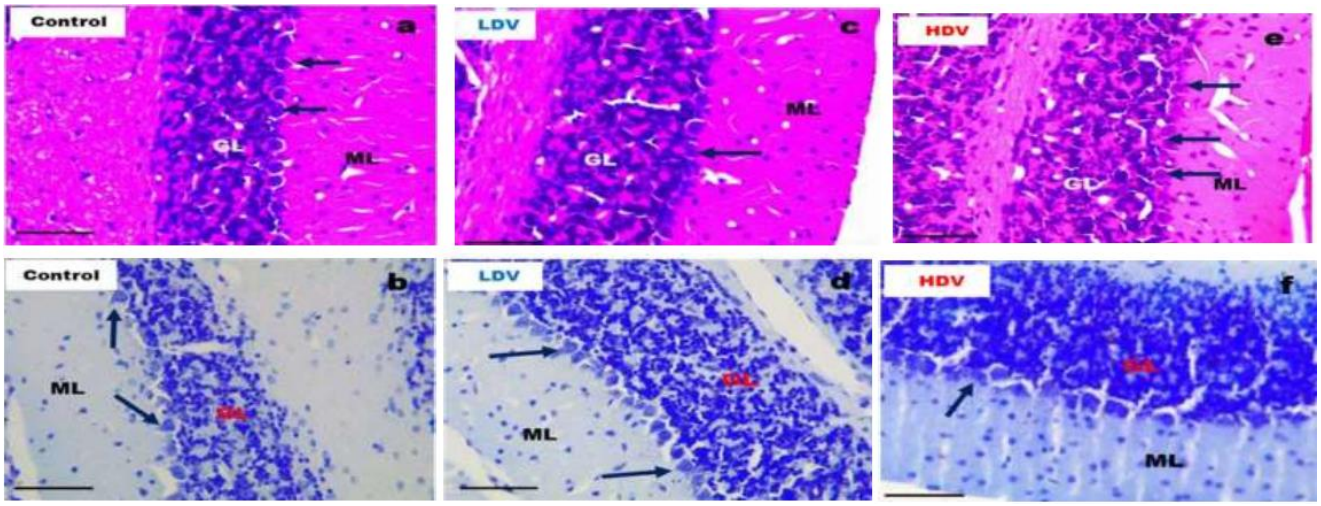

Plate 2:
Photomicrograph of cerebellum from mice. **a-b.** Control group showing normal molecular layer (ML), granular layer (GL) and Purkinje cells (black arrows) **c-d.** LDV group showing apparently normal molecular layer (ML), granular layer (GL) and Purkinje cells (black arrows). **e-f.** Mouse cerebellum from HDV group exhibiting Purkinje cells with pyknotic appearance, loss of Purkinje cells and also loss of dendritic arborization. **a,c,e** H&E staining while **b,d,f** Cresyl Violet staining. x40 Magnification. Scale bar = 100 µm

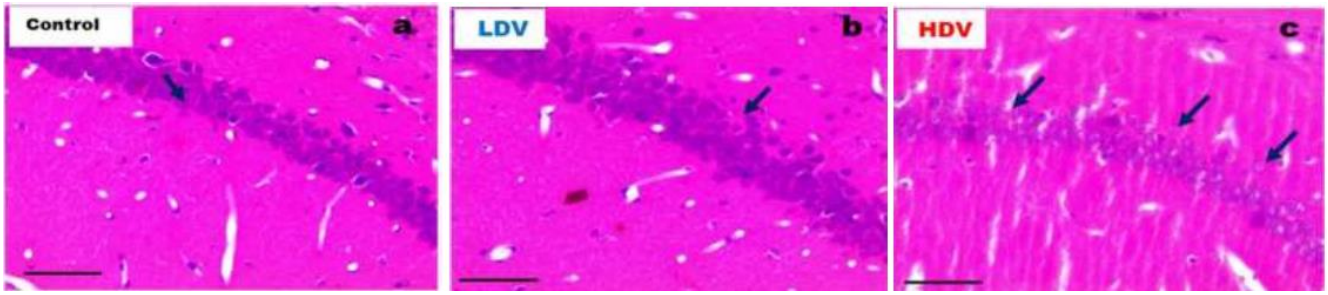

Plate 3:
Photomicrograph of hippocampus showing the *cornus ammonis* 1 (CA1) in mice. **a.** The control group showing normal CA1 cells with dendritic extension. **b.** LDV group also showing normal pyramidal cells. **c**. The CA1 showing multiple apoptotic death neurons and the neuronal morphology appear distorted with reduced neuronal cells. H&E staining. x40 Magnification. Scale bar = 100 µm

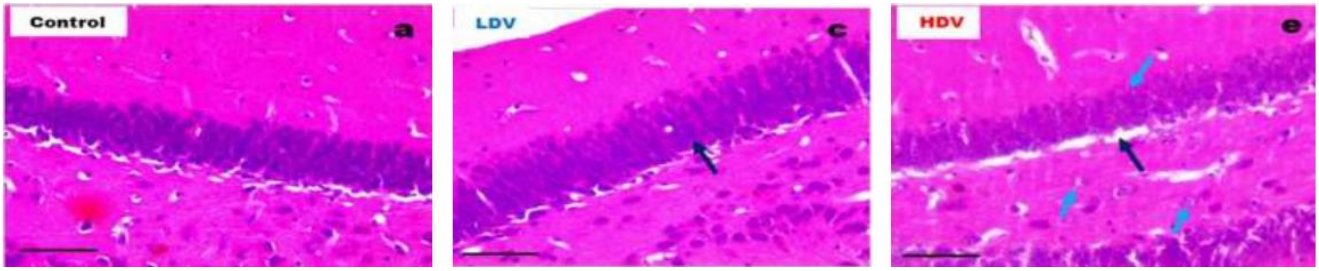

Plate 4:
Photomicrograph of hippocampus showing the dentate gyrus in mice. **a**. The control group showing normal dentate gyrus cellular architecture (H&E staining). **b.** The control group showing normal dentate gyrus cellular architecture (Cresyl violet staining). **c.** LDV group also showing normal dentate gyrus (H&E staining). **d.** LDV group also showing relatively dentate gyrus with mild vacuolation (Cresyl violet staining) **e.** The dentate gyrus in the HDV exhibiting multiple deformed neurons (blue arrow) and vacuolation (black arrow) (H&E staining). **f.** In HDV group, we saw also deformed neurons and vacuolation (black arrow). (Cresyl violet staining). x40 Magnification. Scale bar = 100 µm.

Examination of the kidneys in the control group mice revealed normal glomeruli, distal, proximal tubules in the cortex and the medulla. The kidney in LDV mice also had normal glomeruli, but mild sloughing off of nephron tubules and normal medulla. The group exposed to HDV exhibited renal glomerular shrinkage and some of the renal tubules had abnormally larger lumen compared to the control and LDV groups. There was also the presence of degenerated epithelial cells in the renal medulla of mice exposed to HDV

Mice in the control group presented normal hepatic cells, arranged in distinct sinusoids. The LDV group also had normal hepatic cells, but with mild sinusoidal dilatation. Furthermore, there was moderate congestion in the blood vessels of the liver and the hepatocytes showed diffused vacuolar degeneration in the HDV group.

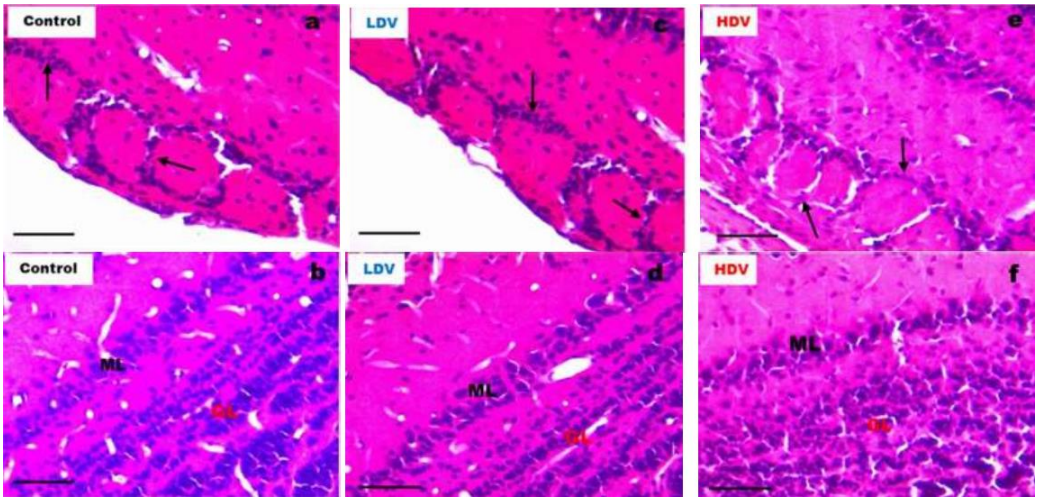

Plate 5:
Photomicrograph of the olfactory bulb in mice. a. Showing normal olfactory glomerular cells b. Control group also showing normal mitral cells and granular cell layer of olfactory bulb. c. The LDV group showing normal olfactory glomerulus. d. The LDV group showing normal mitral layer and normal granular layer. e. The HDV showing olfactory glomerular cells which are undergoing apoptosis and the glomerular cells are fewer in number. f. The mitral cell layer showing fewer cells and are undergoing apoptosis in HDV group with distorted granular cells layer. H&E staining. x40 Magnification. Scale bar = 100 µm.

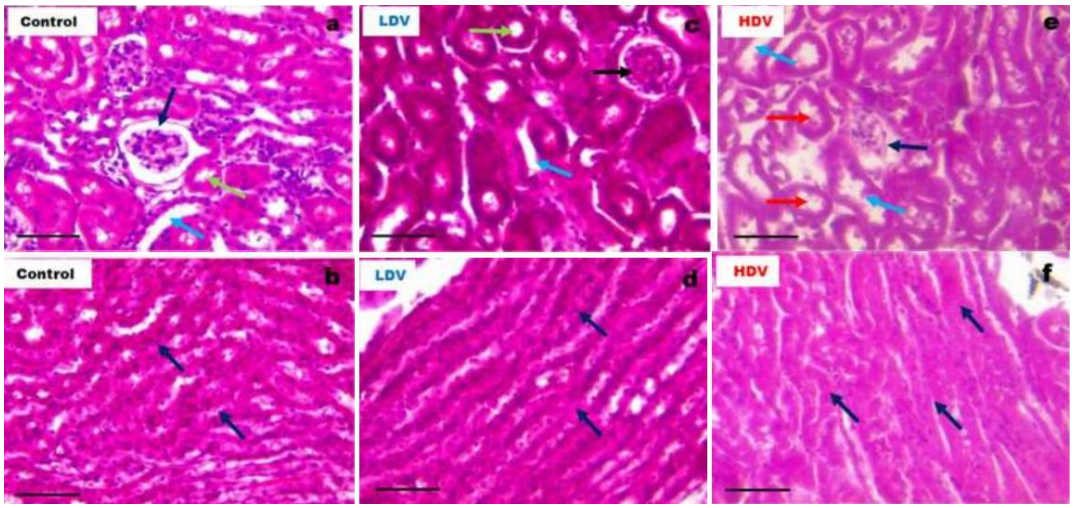

Plate 6:
Photomicrographs showing the effects of low dose and high dose vanadium exposure of on kidney of mice. **a.** Renal cortex of control group showing normal glomerulus distal (black arrow), proximal (red arrow) tubular and glomerulus (white arrow) structures are seen in the medulla of control kidney. **b.** The control group showing normal renal medulla **c.** LDV group also showing normal glomerulus (black arrow) and mild sloughing off of nephron tubules (black arrow). **d.** The LDV group showing normal renal medulla **e.** HDV group showing shrinkage of renal glomerulus (black arrow); some of the renal tubules with larger lumen (blue and red arrows). **f.** Renal medulla of HDV group showing degenerated epithelial cells (black arrow). H&E stain. x40 Magnification. Scale bar = 100 µm

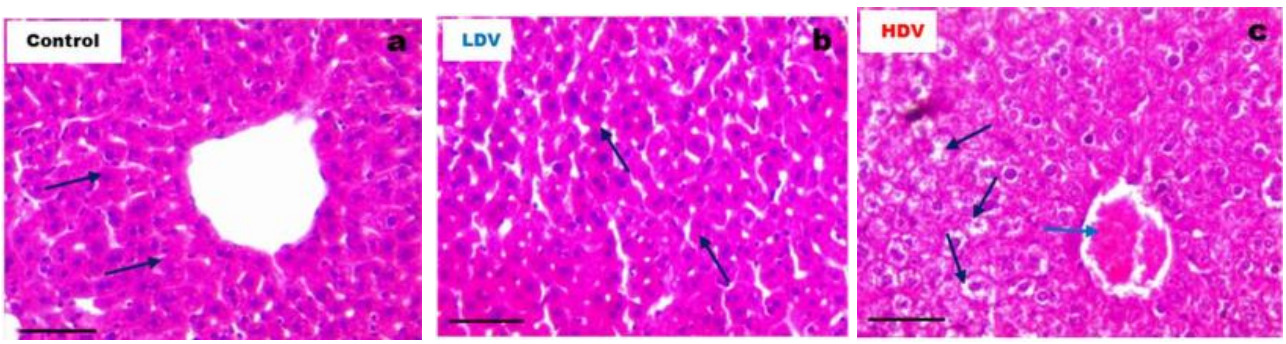

Plate 7:
Photomicrographs of the Liver of mice exposed to low dose and high dose vanadium. **a.** Control group showing normal hepatic cells. **b.** LDV group also having normal hepatic cells with mild sinusoidal dilatation. **c.** Showing diffuse hepatocellular vacuolar degeneration (black arrows) and moderate congestion of blood vessels (blue arrow). H&E staining. x40 Magnification. Scale bar = 100 µm.

DISCUSSION

In this study, we observed that there was significant increase in average body weight of low-dose vanadium exposed mice while average body weight decrease was noted in high-dose vanadium exposed animals in comparison to the control group. This finding, especially for the low-dose vanadium group is similar to the work of Omayone *et al*., (2020). Omayone and his team observed that exposure to low-dose vanadium after 10 weeks in neonatal rat led to significant increase in body weight. Likewise, in a recent report by Dyer and De Butte (2022), they saw significant increase in body weight of low-dose vanadium exposed mice over controls. In addition, Usende *et al*., (2016) reported astrocytosis including oligodendrocytes (immature and mature) proliferation with low-dose vanadium. This demonstrates vanadium's capacity to regulate signal transduction pathways facilitated by growth factors, enhancing transformational changes in cells (Stern *et al*., 1993). On the other hand, Olopade *et al*., (2011), Azeez *et al*., (2016), Usende *et al*., (2016) and Audu *et al*., (2020), have also reported a marked reduction of body weight in neonatal murine species treated with high-dose vanadium as compared to the control and low-dose vanadium groups. Contrary to the average body weight findings obtained for high-dose vanadium group, García *et al*., (2004, 2005), Igado *et al*., (2020) and Folarin *et al*., (2017), exposed adult murine species to high-dose vanadium and they observed no statistical differences in body weight compared to their respective control groups. Thus, we can predict the reason why the current work showed differences as the period for which the animals are exposed to vanadium and variation in age of the rats utilized in the investigations. In their work, they used older murine but as early as postnatal day one our animals were being treated, and we observed variations in the weight as the mice developed. Therefore, both low-dose and high-dose vanadium strongly affect postnatal development.

In this study low-dose vanadium group had a slight observable increase in the relative brain weight while there was a statistically significant decrease in relative brain weight for the high-dose vanadium group compared to the control group. Although, decrease in relative brain weight has been reported by Olopade *et al*., (2011), and Usende *et al*., (2016) in neonatal murine species exposed to high-dose vanadium, there is a scarcity of reports on this same parameter for low-dose vanadium. The increased relative brain weight for low-dose vanadium in relation to control for this study could therefore be regarded the first report of low-dose vanadium positively affecting the brain.

Our study shows improvement in learning and motor functions for low-dose vanadium group. Low-dose vanadium mice had improved hanging strength than control and high-dose vanadium group. This is possible considering insulinomimetic properties of vanadium reported by Semiz and Mcneill (2002), which cause increased glycogen storage in skeletal muscle increasing muscle endurance. Therefore, the improved performance of the low-dose vanadium group in the forelimb grasping in pups and hanging wire test in adult mice is likely to be as result of vanadium-induced increase in glycogen content of the skeletal muscle. Dyer and De Butte (2022), similarly observed improvement in motor activities of chronic low dose vanadium exposed rats. We have also shown that mice with high-dose vanadium exposure had a significant reduction in latency to fall compared to controls. This is similar to report by Folarin *et al*., 2017, who reported reduction in griping strength of vanadium-treated animals relative to control, however, it was statistically insignificant. Additionally, administration of high dose vanadium has previously been reported to result in muscular weakness in mice as reported by Mustapha *et al*., (2014), Azeez *et al*., (2016) and Audu *et al*., (2020).

The Morris water maze measures the hippocampal-dependent spatial navigation and learning which is assessed via multiple trials as well as memory ability to determine the platform area when the platform is removed, usually referred to as probe trial (Morris, 1993). In our study, we observed that mice with LDV-exposure had significantly improved memory capacities than the HDV-exposed mice, but comparable with the controls. Dyer and De Butte (2022), however reported that control rat performed better than rats with chronic low dose vanadium administration (0.05mg of vanadium powder/1000 ml of food mash). Long term high-dose vanadium administration in adult mice has also been previously reported to result in significant memory deficits compared to controls when tested using the Morris water maze (Folarin *et al*., 2016).

Our study observed that the high-dose vanadium group take longer time to reorient themselves against gravity when compared to control and low-dose vanadium exposed mice, however, it is not statistically significant. This finding agrees with Usende *et al*., (2016) who reported that high-dose vanadium treated groups could not make an 1800 turn in a shorter period after placement on inclined platform at PND 15 as well as PND 21 and this was statistically significant. Despite the longer grasping capacity of low-dose treated mice pups compared to control, there was no significant differences. Contrary to our finding in vanadium, exposure of neonatal rats to low concentration (50ppm) of lead over three months resulted in impaired motor functions (Mameli *et al*., 2001). This finding is similar to our observation in high dose vanadium-exposed mice in this study, although, our study showed that high-dose vanadium exposure significantly affect forelimb grasping capacity in the mice pups.

The main organs susceptible to toxicity of vanadium are liver and kidney. These organs are reportedly the principal reservoirs for vanadium to pile up following absorption. (Sabbioni *et al*., 1978; Ramanadham *et al*., 1991; Sanchez *et al*., 1998, Omayone *et al*., 2020). Histological studies in the kidney revealed severe shrinkage of renal glomerulus in the high-dose exposed vanadium. Furthermore, some of the renal tubules have larger lumens while the renal medulla present degenerated epithelial cells in high-dose vanadium exposed mice; in the liver, there was diffuse hepatocellular vacuolar degeneration and moderate congestion of blood vessels among the high-dose exposed mice.

In conclusion, vanadium in low-dose is shown to facilitate increase in body weight which translated also into organ weight, while causing neurodegenerating changes at high-dose. We also discovered that low-dose vanadium exposure improved neurobehavioral/ neurodevelopmental performances whereas high-dose vanadium led to decreased neurobehavioural performances. Furthermore, our results demonstrated that low-dose exposure to vanadium caused

mild histological lesions in some parts of the brain, liver and kidney whereas high-dose showed distinct histological lesions in different parts of the brain, liver and kidney. Additionally, because only basic histological staining was included in our study, future research should include different additional tools such as immunohistochemistry and electron microscopy in other to determine further differences in how both low -dose and high-dose vanadium affects living system.

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Full length Research Article

Vitamin C Supplementation Promotes Locomotor and Exploratory Behaviours in Male Wistar Rats Exposed to Varying Stress Models

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Summary: Constant exposure to environmental stress has negative behavioural outcomes. Considering the inverse relationship between stress and Vitamin C intake, this study was aimed at investigating variable stress techniques and Vitamin C supplementation on exploratory/locomotor behaviors in male Wistar rats. Twenty-eight male Sprague-Dawley rats (100g-120g) were allotted into four groups (n=7). Control received 10ml/kg distilled water, group two received 100 mg/kg vitamin C, group three was exposed to different models of stress while group four was stressed alongside 100 mg/kg vitamin C. Vitamin C treatments were given orally for 2 weeks. Animals in groups 3 and 4 were stressed every other day with models such as multiple cage changes, exposure to noise, overnight strange objects, overnight wetting of beddings, and immobility. Explorative and locomotor activities were assessed with the open field test, novel object recognition test, and Y maze test using a Logitech camera and ANY-maze software to track the movement of the rats. Cortisol was assayed in the serum using Enzyme-linked Immunoassay (ELISA) kit. Superoxide Dismutase, catalase, and lipid peroxidase; malondialdehyde (MDA) were also assayed in the serum. Results were analyzed using graphed prism 5.0, analysis of variance (ANOVA) was used to compare between groups while the mean with $P<0.05$ were considered significant The results show that locomotor activities such as distance traveled, average speed, and time spent in the center square was significantly reduced by stress. These activities were improved with the intake of vitamin C compared with stress. Explorative activities such as locomoting around the environment, orientating towards novelty, and touching or sniffing novel objects were significantly increased in the rats on Vitamin C supplements and reduced in the stressed group. In the serum, cortisol level was significantly increased in rats exposed to stress and decreased with Vitamin C intake. Stress also significantly increased MDA and decreased SOD and CAT while vitamin C supplement decreased MDA and increased SOD and CAT. In conclusion, oral intake of vitamin C enhanced explorative/locomotor behavior and increased oxidative stress in rats exposed to different models of stress.

Keywords*: Stress, Vitamin C, Explorative Behavior, Locomotor Behavior, Oxidative stress*

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INTRODUCTION

Stress is the body's response to an environmental demand of conditions that outweighs an individual's psychological and physical ability to cope effectively (Crosswell & Lockwood 2020). The change caused by stress may be due to an individual's environmental sources or internal acuities. Other than being an emotion, stress is a physical response that travels throughout the entire body. It has an advantage when it is for a short term but when activated too often it increases the risk of biological, social, and psychological problems (Tucker *et al*., 2008). These problems, therefore, increase the risk of developing cardiovascular disease, depression, anxiety symptoms, migraine, sleep, and appetite alterations among others (Marik 2020). In addition, the stress response is necessary for preserved evolutionary adaptation which is instrumental in the enhancement of human complexity (Edwards *et al*., 2008). This complexity contributes to survival (Tucker *et al*., 2008) by evoking lasting behavioral and body changes (Blossom *et al*., 2020). Rodents mimic the same pathophysiological and behavioral changes during stressful situations as humans (Jaggi *et al*., 2011). A rodents-based behavioral test can detect changes in behavioral patterns in animals. When faced with an unfamiliar environment or object in an open-field test (OFT), these animals often exhibit behavioral patterns that can be termed exploration, such as locomoting around the environment, orientating towards novelty, and touching or

sniffing novel objects. This procedure provides an animal model of anxiety-like behavior that permits the evaluation of different aspects of animal behavior (Zimcikova *et al*., 2017). The number of lines crossed, the frequency of rearing and grooming are measures of locomotive behaviors and index for the rodent's emotion. Aside from these, distance covered, speed, the number, duration, and time spent in Central Square are also regarded as measures of exploratory behavior and anxiety (Zimcikova *et al*., 2017).

In addition, grooming in rodents is a complex and ethologically rich behavior that is sensitive to stress and various genetic and pharmacological manipulations which may alter gross activity as well as patterning (Smolinsky *et al*., 2009). Rearing also shows exploratory capacity which has consequences of neuronal dysfunction following manipulation (Idowu *et al*., 2019). Therefore, observational analysis of these activities serves as a useful measure of stress and anxiety in both wild and laboratory animals (Smolinsky *et al*., 2009).

According to Sharma *et al*. (2022), oxidative stress is a major cellular burden that triggers reactive oxygen species (ROS) and deteriorates antioxidants. The high oxygen consumption in the by-products of increased metabolic rate cause toxicity of transition metals that catalyze the production of reactive oxygen species. Thereby causing the accumulation of ROS to exceed the ability of antioxidants that neutralizes them (Moussa *et al*., 2019). The outcomes of oxidative stress (OS) include lipid peroxidation, oxidative damage to DNA and proteins as well as alteration of the antioxidant enzymes response (Aboul-Ela *et al*., 2011). Lipid peroxidation has been used successfully as a measure of oxidative stress because of the capability of free radicals in generating lipid peroxidation processes in organisms. However, malondialdehyde (MDA) is a final product of polyunsaturated fatty acids peroxidation in the cells and a marker generally acceptable for lipid peroxidation (Gaweł *et al*., 2004).

The toxic cause OS and polyunsaturated fatty acids which are free radicals seen in oxidative stress are linked with a high rate of oxygen consumption alongside low levels of endogenous antioxidants (Moussa *et al*., 2019). These endogenous antioxidants can be enzymatic and non-enzymatic. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) are enzymatic antioxidants which serve as the first line of defense against oxidative stress (Aguilar *et al*., 2016). The non-enzymatic antioxidants are vitamins E, A, and C, flavonoids, carotenoids, glutathione, plant polyphenols, uric acid, theaflavin, allyl sulfides, curcumin, melatonin, bilirubin, and polyamines (Mirończuk-Chodakowska *et al*., 2018).

The animals were supplemented with vitamin C (L-ascorbic acid) which is an optically-active hydro-soluble free radical scavenger and an essential nutrient usually obtained through diets (Moussa *et al*., 2019). However, it is an important vitamin that participates in numerous cell functions. Our body is known to produce antioxidants but during stress, the antioxidants produced become inadequate to scavenge free radicals (Salami *et al*., 2020). According to Blossom *et al*. (2020), stress increases the chances of neurological disorders in animals and humans (Marik 2020) However, there is still a need to explore the combined effects of the common antioxidant; vitamin C on behavioral activities during stress. This study was therefore designed to investigate oral intake of vitamin C on behavioral outcomes in rats exposed to stress.

MATERIALS AND METHODS

Animals: Twenty-eight male Wistar rats (100-120g) were obtained from the animal house at Lagos State University College Medicine, Ikeja. The rats were kept in the animal house at room temperature and a natural light rhythm of 12 hours of daylight and 12 hours of darkness. They were acclimatized for 2 weeks and provided with rat chow and water ad libitum. The research protocol was approved by the Lagos State University College of Medicine Ethics and Research Committee (Ref. No; AREC/2012/016).

Drugs: The Vitamin C used in this study was purchased from Sigma Chemical Co. St. U.S.A. Normal saline purchased from Unique Pharmaceuticals, Sango-Otta, Nigeria.

Experimental Design: The rats were randomly divided into four groups (n=7), control received 10ml/kg BW/day distilled, group II received 100mg/kg BW/day Vitamin C (Adeneye and Olagunju 2008) group III were subjected to stress regimen alongside 10ml/kg BW/day distilled water while group 4 were also stressed and received 10 mg/kg BW/day Vitamin C. Both treatment and stress were done for 2 weeks and the following parameters were carried out across the group.

Stress models: Rats were exposed to different stress regimens of the light and dark cycle according to Borrow *et al*., (2018) every other day for 2 weeks. The beddings were wet overnight (dark cycle) by filling the cages with 700 ml of water. Strange objects (marbles) were kept in the cages overnight and removed in the morning (dark cycle). Multiple cage change during the light cycle was done by transferring the rats from one cage to another at an interval of ten minutes for 2 hours. The rats were restrained by keeping the animals in a cylindrical or semi-cylindrical tube with ventilation holes for 30 min. Immobilization models produce inescapable physical and mental stress with a low rate of adaptation. A noise disturbance was induced by using loudspeakers (15W) connected to a white noise generator (0-26 kHz) which is located 30 cm from the cage. The noise was set at 100 decibels. The animals were exposed to this noise protocol for 4 hours/per day.

Open Field – (Assessment of Locomotor Activity and Exploratory Behavior):

Locomotor activity and explorative behaviors were conducted in an Open Field (OF) box (40cm x 40 cm) according to Idowu *et al*. (2019) A Logitech camera (C270, UK) was connected to a computer with ANY-maze behavioral tracking software (Stoelting Co., USA). The camera was placed on the box for easy viewing of the boundaries and dimensions of the box. In line with Idowu *et al*. (2019) the floor of the box was divided into sixteen dimensions of 10cm X 10cm squares using the ANY-maze protocol. These lines were used to track the animals based on the amount of time spent in the corners and the number of times spent in the Centre zone of the box.

For the locomotor behavior, the total distance traveled, average speed in the box, as well as the number of lines crossed during the test, was assessed. In explorative behavior, the amount of time spent in the corner square was considered.

Novel Object Recognition Test: The object recognition test (ORT), also known as the novel object recognition test (NORT) is used to evaluate cognition as well as learning and memory because it is less stressful for rodents (Sik *et al*., 2003). The test relies on three sessions: habituation, training, and test session.

The habituation was done for 5 minutes in the apparatus which is the same box used for the open-field experiment prior to the NORT. This is followed by two stages (training and test session) for the novel object recognition task after 24 hours. During habituation, the rat was placed in the NORT box and allowed to freely explore the arena for 5 min. Training sessions were done after 24 hours of the habituation. Two identical objects were placed in opposite quadrants of the arena (i.e., NE corner and SW corner) equidistance from one another. The rat was then placed in the center of the arena, equidistant from the 2 identical objects, and was allowed free exploration for 5 min. At the end of the trial, the rat was removed and placed in the holding cage. After 15 minutes of the training session, the test session was done. Each rat was placed into the NORT box which has a familiar object (one of the objects from the training session) and a novel object, which was not presented during the training session. If the animal remembers the familiar object from the training session, it should spend more time investigating the novel object during stage 2. The novel object was the same for all the animals used for the study. Logitech camera (C270, UK) was also connected to a computer with ANY-maze behavioral tracking software. The behaviors were viewed, scored, and analyzed by the ANY-maze Behavioural Tracking System (Stoelting Company, USA) software. The camera was connected to a computer where behavior was viewed, scored, and recorded.

The rats were carried to the test room in their home cages and tested for the behavioral tasks individually. Each rat was moved from their home cage to the testing apparatus using a small platform that the rats can comfortably rest on. After every 5 minutes test, each rat was returned to the home cage and the OF apparatus was cleaned with 70% ethanol (ethyl alcohol) and allowed to dry between trials to remove any olfactory clues in the test box. This procedure was used for all the rats tested. The behavioral measures scored during the NORT (Podhorna *et al*., 2002) include:

Line Crossing: The frequency with which the mouse crossed from the square divisions in the open field box

Rearing: The frequency with which the mouse stood on their hind feet or against the wall in any part of the box

Grooming: The frequency and duration of time each mouse spent licking or scratching itself while stationary.

Serum sample collection: Blood samples were taken through a cardiac puncture technique following an injection of 30 mg/kg phento barbital (Salami *et al*., 2020). The samples were collected into a plain bottle using a 5 ml syringe with a needle. The blood was allowed to stand at room temperature for 15–30 min. Thereafter, it was centrifuged using a cold centrifuge. The serum was pipette into a plain bottle and stored at -4Oc

Determination of serum superoxide dismutase, catalase, and malondialdehyde activity

Superoxide dismutase activity in the serum was determined according to methods by Sun and Zigman (1978). This activity is determined by the ability of the enzyme to inhibit the autoxidation of epinephrine while the enzyme activity is monitored at an absorbance level of 480nm. The concentration was expressed as SOD unit/mg protein such that one unit is defined as the amount of enzyme needed to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25◦C and pH 7.8

Catalase activity was determined according to the method of Aebi *et al*. (1984). This is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H202 with the formation of perchromic acid as an unstable intermediate.

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method described by Buege and Aust (1978).

Statistical analysis: The results were expressed as mean ± SEM (standard error of mean). Statistical analysis was done using GraphPad Prism (version 5.0) software. One-way analysis of variance (ANOVA) was used to compare between groups while Newman keuls was used as post hoc test. Values with $P<0.05$ were considered significant.

RESULTS

Total distance traveled in the open field (OF) box for a period of 300 seconds (5 minutes): The distance traveled in the open field box shows that the stressed rats ($P\leq0.001$) and rats on Vitamin alongside stressed ($P\leq0.01$) traveled less distance when compared with the control. Distance traveled was increased in rats on vitamin C ($P\leq0.05$), ($P\leq0.001$) when compared with the control and stressed group (Figure 1).

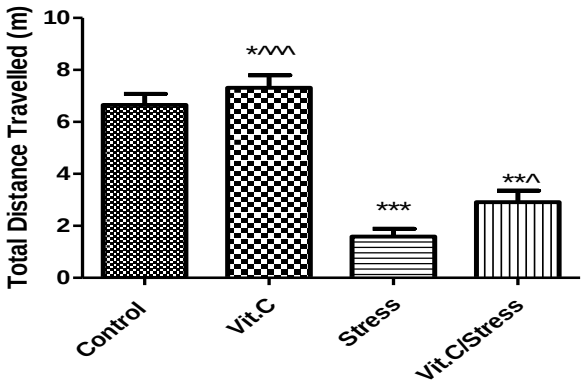

Fig. 1:
Total distance travelled in the open field (OF) box for a period of 300 seconds (5 minutes)
Key: ***,**,*=P<0.0001, P<0.001, P≤0.05 when compared with control, ^=P≤0.05 when compared with stress.

Average speed in the open field (OF) box for a period of 300 seconds (5 minutes): Average speed in the open box was reduced in the stressed animals ($P\leq0.01$) as well as

animals in the Vitamin C/stressed group (P≤0.05) when compared with the control. The average speed was higher in Vitamin C (P≤0.05) than in the control. It was also higher in Vitamin C/stress (P≤0.05) as well as in the Vitamin C (P≤0.01) group when compared with the stress group (Figure 2).

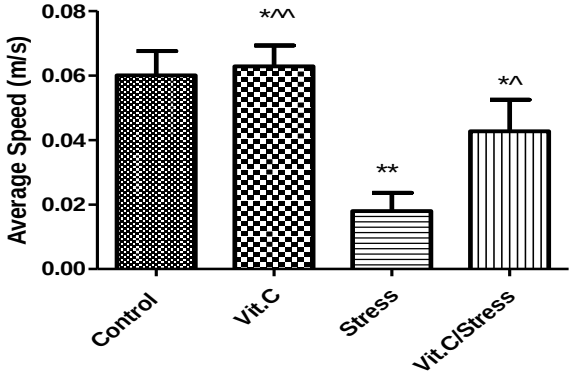

Fig. 2:
Average speed in the open field (OF) box for a period of 300 seconds (5 minutes)
Key: *=P≤0.05, **=P≤0.01 when compared with control, ^=P≤0.05, ^^= P≤0.01 when compared with stress.

Number of entries into the center zone in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: Centre zone entry by Stress (P≤0.01) and Vit.C/Stress (P≤0.05) group was reduced when compared with the control. There was an increase in the rearing episodes in the Vit. C (P≤0.01) and stress/Vit. C (P≤0.05) when compared with stress (Figure 3).

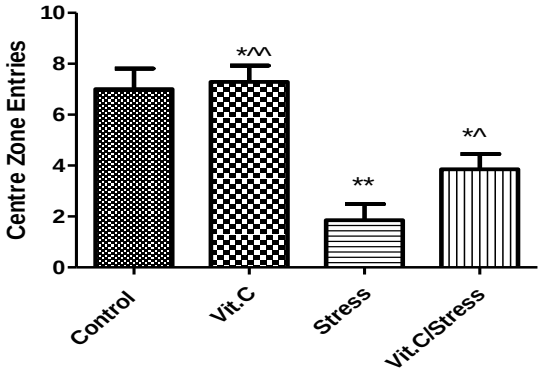

Fig. 3:
Number of entry into the center zone in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P≤0.05, **=P≤0.01 when compared with control, ^=P≤0.05, ^^=P≤0.01when compared with stress

Total time inactive in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: The total time inactive in the stress (P≤0.001) and Vit.C/Stress (P≤0.01) group was reduced than in the control. It was also lower in stress/Vit. C (P≤0.001) and Vit. C. (P≤0.05) compared with the stress group (Figure 4).

Total inactive Episodes in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: The episode of inactiveness was higher in stress (P<0.05) when compared with the control. These episodes were lower in Vit. C (P<0.05) as well as Vit.C/Stress (P<0.01) groups than in control. (Fig. 5).

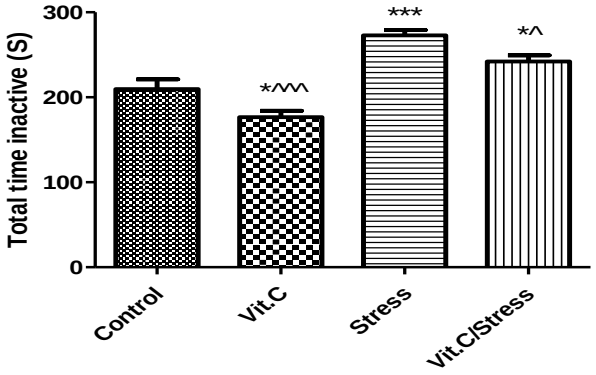

Fig. 4:
Total time inactive in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P≤0.05, ***=P≤0.001 when compared with control.^=P≤0.05, ^^^=P≤0.001 when compared with Stress

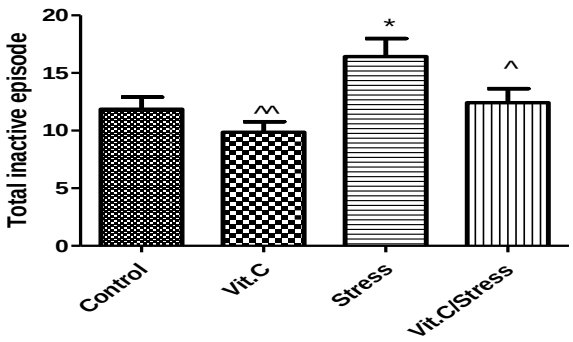

Fig. 5:
Total inactive episodes in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P<0.05 when compared with control, ^=P<0.05, ^^=P<0.01 when compared with Stress,

Episodes of Grooming in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: Grooming episodes were significantly increased in the stress (P<0.05) when compared with the control. There was a significant decrease in grooming episodes in Vit C (P<0.05) and Vit. C/Stress (P<0.05) group when compared with stress. (Figure 6)

Grooming time in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: Grooming episodes were significantly increased in the stress (P<0.05) when compared with the control. There was a significant decrease in grooming episodes in Vit C (P<0.05) and Vit. C/Stress (P<0.05) group when compared with stress. (Figure 7).

Episodes of Rearing on the wall in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: The episodes of rearing on the wall in the stress group (P<0.05) were lower and higher in Vit. C (P<0.05) group than in the control. There was an increase in rearing

episodes in Vit. C (P<0.001) and Vit. C/Stress (P<0.05) group when compared with stress. (Figure 8)

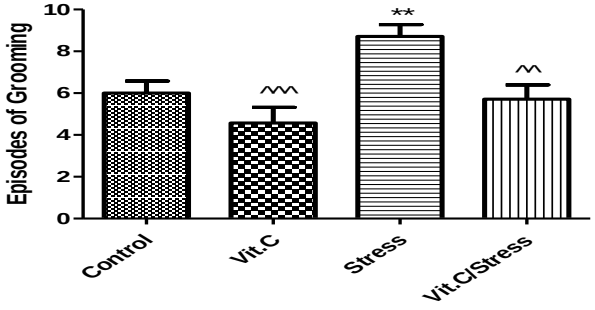

Fig. 6:
Episodes of Grooming in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: **=P<0.05 when compared with control, ^^=P<0.01, ^^^= P<0.001 when compared with Stress.

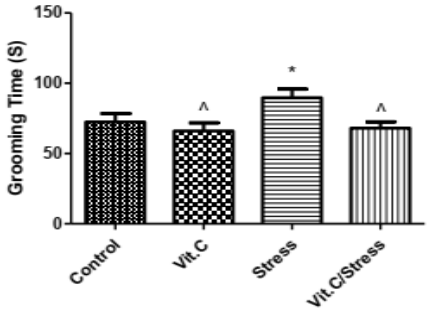

Fig. 7:
Grooming time in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P<0.05 when compared with control, ^=P<0.05 when compared with Stress**.**

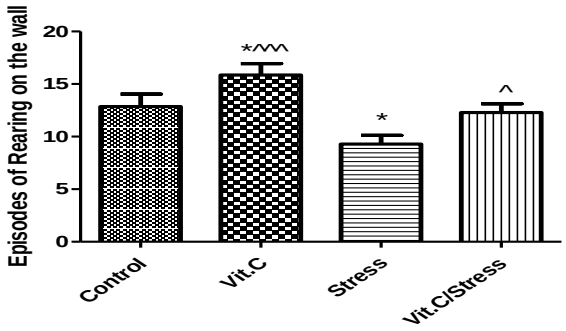

Fig. 8:
Episodes of Rearing on the wall in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P<0.05 when compared with control, ^=P<0.05, ^^^= P<0.001 when compared with Stress.

Episodes of Rearing in the air in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: The episodes of rearing in the air in the stress group (P<0.05) were lower and higher in Vit. C (P<0.05) group than in control. There was an increase in rearing episodes in Vit. C (P<0.01) and Vit. C/Stress (P<0.05) group when compared with stress. (Fig. 9).

Episodes of Rearing on familiar object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats: The episodes of rearing on the novel object were lower in the stress group (P<0.05) and higher in Vit. C (P<0.05) group than in the control. Rearing was also higher in Vit. C (P<0.001) and Vit. C/Stress (P<0.01) group when compared with stress. (Fig. 10)

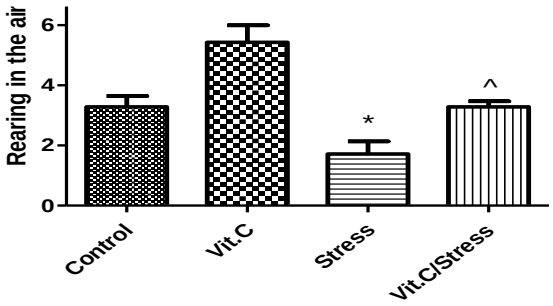

Fig. 9:
Episodes of Rearing in the air in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P<0.05 when compared with control, ^=P<0.05, ^^= P<0.01 when compared with Stress

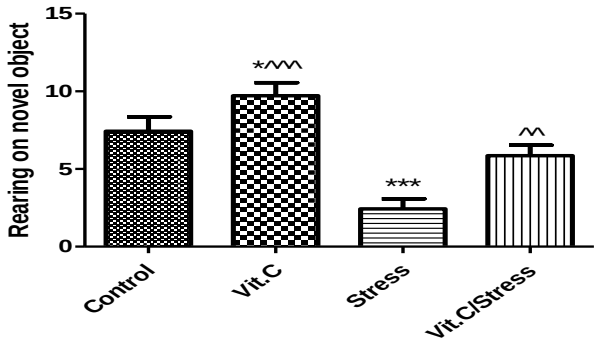

Fig. 10:
Episodes of Rearing on novel object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
Key: *=P<0.05, ***=P<0.05 when compared with control, ^^, ^^^= P<0.01, P<0.001 when compared with Stress.

Episodes of Rearing on familiar object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:
The episodes of rearing on the familiar object were higher in the stress group (P<0.05) than in the control. Rearing on the familiar objects was also lower in Vit. C (P<0.05) as well as Vit. C/Stress (P<0.01) group when compared with stress. (Fig. 11).

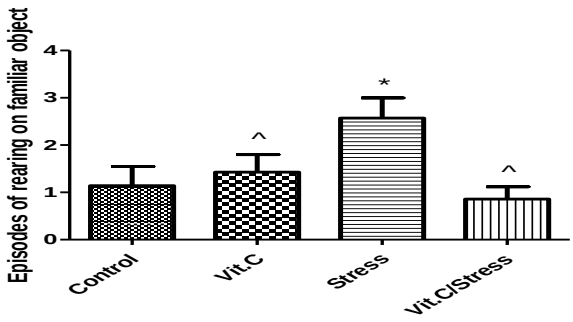

Fig. 11:
Episodes of Rearing on familiar object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats
*Key: *=P<0.05 when compared with control, ^=P<0.05 when compared with Stress.*

Cortisol, MDA, and SOD on Vitamin C and stress in Wistar rats: Serum level of cortisol was high in the stress group (P<0.05) when compared with the control. It was lower in Vit. C and Stress/Vit.C, (P<0.05) than the stress group while stress/Vit. C was significantly higher (P<0.05) than Vit. C (table1)

In serum Malondialdehyde there was a decrease in stress (P<0.01) and no significant difference in Vit. C and stress/Vit. C when compared with control. There was a significant decrease in the MDA level in Vit. C (P<0.001) and Stress/Vit. C (P<0.05) when compared with the stress group. It was increased in the stress/Vit. C when compared with Vit. C

There was a decrease in the serum SOD in stress (P<0.01) and an increase in Vit. C when compared with control. In Vit. C (P<0.001) and stress/Vit. C (P<0.001) group, SOD was increased when compared with stress. It was also increased in stress/Vit. C (P<0.001) when compared with stress (table 1).

Table 1:
Cortisol, MDA and SOD on Vitamin C and stress in Wistar rats

ASSAY/ Group	Control	Vit.C	Stress	Stress/ Vit.C
Cortisol (µg/dl)	1.20 ±0.33	0.88 ±0.24^^	2.58 ±0.22*	2.10 ±0.40^{ns} ^
MDA (**nmol/ml**)	1.34 ±0.18	0.83 ±0.33*^^^	2.15 ±0.19**	1.75 ±0.07^
SOD (**mg/ml**)	2.08 ±0.22	3.08 ±0.22**^^	0.99 ±0.16**	2.03 ±0.20^^

DISCUSSION

In this present study, animal exposure to varying stress showed reduced locomotor activities which were identified by the distance travelled, average speed and time spent in the center square. According to Sgroi *et al.* (2014) locomotor activity assessed by the open field test (OPF), accounts for the spontaneous exploratory behavior of animals in defining a precise time course of their motor activities. The decreased locomotor activity recorded in this study is in line with previous studies in rats and mice exposed to an acute or chronic stressor (Meerlo *et al.*, 2002) Locomotion is one of the essential functions for animal survival located within the cervical and lumbar regions of the spinal cord. According to Oueghlani *et al.* (2018), the central pattern generators (CPGs) for locomotion are under the control of the supraspinal center. Usually, the CPGs arrange the rhythmical activation of motor neuronal populations which innervates the axial and limb muscles for specific gait patterns involved in locomotor behavior. The increased inactive time and episodes in the stressed rats is a sign that the animals have a decreased ability to explore.

Evidently, the decreased exploratory behaviors show that stress has a significant influence on behavioral patterns in rats. In an open field, the level of locomotion and time spent in the center of the arena are measures of exploratory behavior (Zimcikovaa *et al.*, 2017). The reduction in locomotive activity shows a lack of motivation in the stressed rats. This was accompanied by a decrease in exploration which also shows the lack of interest of the animals in exploring their environment. According to Mällo *et al.* (2007), exploratory behavior by novel stimuli is supported by behavioral as well as postures which allow information about the environment. This information is highly essential for survival due to the possibilities it provides to be able to find food, water, mating partner, shelter, etc.

Furthermore, exploratory behavior is also influenced by the animal's ability to explore a potentially dangerous novel environment or to stay within secure and familiar surroundings. These are indications that an animal's behavior in a novel environment is influenced by curiosity or the motivation to explore (Mällo *et al.*, 2007)

Grooming and rearing are also common behaviors in rats (Kozler *et al.*, 2017) these behaviors and the motor activity involved are used in analyzing impaired behavioral functions in the central nervous system (Kozler *et al.*, 2017). Importantly, grooming is similar across species as well as human thus, it is an innate behavior and a way animal maintains the cleanliness of their body surfaces (Rojas-Carvajal *et al.*, 2022). It has physiological importance such as thermoregulation, social communication, and de-arousal (Kalueff *et al.*, 2016) However, excessive grooming become pathological in some behavioral disorders (Kalueff *et al.*, 2016). The increased grooming episodes observed in our study show the importance of grooming as an adaptive response to stress management (Mu *et al.*, 2020). Grooming is reported in animals with increased corticotropin-releasing hormone (CRH) (Matisz *et al.*, 2021). Thus, corticotropin-releasing hormone stimulates the secretion of pituitary adrenocorticotropin (ACTH) which increases the secretion of cortisol from the adrenal gland (Lightman *et al.*, 2021)

Rearing on the hand is another novel exploratory behavior that assesses the mental and emotional impairment in rats. Generally, the hippocampus is a major control of exploratory behaviors in animals and the key target in stress response. Some component areas in the brain such as basal ganglia, brain stem and cerebellum, limbic system, including the hypothalamus, amygdala and orbitofrontal cortex are also involved in explorative behavior (Kalueff *et al.*, 2016).

Furthermore, rearing is also one of the ways animal behaviors are explored and according to Borta and Schwarting (2005), low rearing activities in rodents are considered as signs of neurologic disorders. In this study, the episodes of rearing were decreased in the stressed animals, however, this has also been reported to be a sign of disturbed motor activity (Kalueff *et al.*, 2016). The low exploratory and locomotor activities observed in the stressed rats are attributed to the high cortisol level in the serum. However, high level of cortisol is mediated by stimulation of the adrenal cortex which is regulated by the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (SNS) (Matisz *et al.*, 2021). Although stress is an important factor in the etiology of behavioral disorders, it also increases the availability of energy for motor activities by raising blood glucose levels (Nelson 2005)

In this present study, oral intake of vitamin C (Ascorbic Acid) increased locomotor activities and also improved the exploratory activities in the rats. In 2020, Moritz *et al.* (2020) reported that ascorbic acid has neuroprotective effects whose mechanism of action is poorly understood. The major route of Vitamin C to the CNS is from the plasma

to the CSF across the epithelium of the choroid plexus (Angelow *et al*., 2003) and it enters the brain interstitium by diffusion (Harrison & May 2009). According to Moritz *et al*. (2020), ascorbic acid also modulates neurotransmitter systems such as aminergic (dopamine), norepinephrine, serotonin (5-HT), glutamatergic and cholinergic systems in the central nervous system. It serves as cofactors in several enzymes (e.g., dopamine B-monooxygenase or prolyl 4-hydroxylase and lysyl hydroxylase (Padayatty & Levine 2016). Evidently, vitamin C is an essential antioxidant in the brain that protects the components of the cells against free radicals formed during metabolism (Padayatty & Levine 2016). The constant use of oxygen in burning metabolic fuel for energy during normal cellular metabolism increases the formation of free radicals (Srivastava & Kumar 2015). Therefore, incessant exposure to stress will further increase metabolic rate and ultimately generates more free radicals that becomes toxic to the system.

According to Padayatty & Levine (2016), the inverse relationship between Vitamin C and stress in most animals provides an evidence of the significance of Vitamin C in the outcomes of stress. In most mammals, Vitamin C is synthesized in the liver from glucose-6-phosphate and from fructose-6-phosphate in plants (Marik 2020). In contrast, human, rodents and animals such as teleost fish, guinea pigs, and a few species of bats do not synthesize vitamin C due to evolutionary loss of function mutation in the L-gluconolactone oxidase (Gulo). The enzyme L-gluconolactone oxidase (Gulo) catalyzes the last rate-limiting step in the biosynthesis of vitamin C. Therefore, vitamin C must be supplied through dietary sources and supplements in humans and rodents (Mustafi & Wang 2020).

According to Khassaf *et al*. (2003) Vitamin C directly reduces oxidative stress by reducing the free radical species generated from the H2O2. However, the increased SOD and CAT are evidence of the stimulation of SOD and CAT transcription which is highly sensitive to the redox state of cells. Therefore, oral intake of Vitamin C was able to maintain the compromised cellular environment which is the likely cause of the neurogenic disorders caused by stress.

In conclusion, exposure of rats to a variety of stress increased serum cortisol levels and oxidative stress. Locomotor and explorative activities in the rats are altered with exposure to varying stress. However, with oral intake of Vitamin C, these neurogenic activities altered by stress were improved in the rats.

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Full length Research Article

Effect of Standardized *Eucalyptus globulus* Leaf Extract on Brain Oxidative Stress and Aberrant Neurochemistry of Fructose-streptozotocin-induced Diabetic Rats.

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Summary: The neuro-pharmacological effect of *Eucalyptus globulus* ethanol leaf extract in fructose-streptozotocin-induced diabetic rats was evaluated in this study. The phytochemical analysis of the extract was carried out using HPLC-DAD. Diabetes was induced in rats with 10% fructose in drinking water and a single intraperitoneal injection of 40 mg/kg streptozotocin (STZ). Diabetic animals were orally treated with 100-400 mg/kg of the extract for 21 days with glibenclamide as the reference drug. Blood and brain tissue were processed for the determination of serum electrolyte levels, hematological indices, and biochemical estimations. Ergosterol, pinitol, catechin, quercetin, robinetinidol, and other polyphenols were identified in the extract. Diabetic animals showed decreased serum potassium and sodium ion levels and decreased hematocrit, hemoglobin, red blood cells, white blood cells and lymphocytes but increased neutrophils. The brains of animals in the untreated diabetic group with increased blood glucose level showed oxidative stress (increased level of MDA and myeloperoxidase but decreased level of reduced glutathione and superoxide dismutase) and disturbed neurochemistry (increased level of acetylcholinesterase and monoamine oxidase but decreased level of Na+K+ ATPase, tyrosine hydroxylase and dopamine). Administration of the *Eucalyptus globulus* leaf extract remarkably ameliorated the observed hyperglycemia, electrolyte, and hematological imbalances in animals. In addition, the administration of the extract attenuated the brain redox imbalance and neurochemical disturbances in the rats. These results show that *Eucalyptus globulus* leaves contain antioxidant and neurotransmitter-modulating phytochemicals with the potential to be developed as therapeutic agents for the management of diabetic cerebrovascular problems and related complications.

Keywords*: Type-2 diabetes; hematological disturbances; brain redox stress; neurotransmitter dysfunction; phytoextract*

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease burden related to diabetes is high and has continued to rise in every country, fuelled by the global rise in the prevalence of obesity and unhealthy lifestyles such as poor diets and physical inactivity. The two common types of diabetes mellitus are type 1 and type 2 diabetes mellitus. Type-2 diabetes represents over 90% of all cases of diabetes (Obafemi *et al*., 2017).

Diabetes mellitus (DM) and its complications pose a major threat to global health. The International Diabetes Federation (IDF) estimated in 2019 that 1 in 11 adults aged 20–79 years (463 million adults) had diabetes mellitus globally and this is projected to rise to 700 million by 2045, fthe largest increase is projected to come from Africa with an estimated increase of 143% (IDF, 2019). Diabetes is estimated to be associated with 11.3% of global deaths from all causes among people in this age group (IDF, 2019). DM and its complications impose an enormous health and economic burden upon individuals, families, societies and nations (Li *et al*., 2019).

Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs (Fasil *et al*., 2018). Hyperglycaemia is associated with several physiological changes, and the most profound effects are seen in the brain, where glucose is the major substrate for energy metabolism and both the local energy store and the supply of alternative sources are limited. Neurotransmitters have a role in maintaining glucose homeostasis (Güemes & Georgiou, 2018). Brain injury results from a derangement of several biochemical processes in the organ that are initiated when blood glucose concentration is altered. It is also known that the expression of some genes is involved in the pathophysiology of diabetic neurological dysfunction, but the action mechanisms are often obscure.

Although several drugs are available to control elevated blood glucose levels in diabetic patients, they still suffer from treatment complications like nephropathy,

neurological dysfunction, and retinopathy. Hence, the search for new treatment strategies is required to manage diabetes and mitigate these chronic widely spreading complications. Medicinal plants and plant-derived antioxidant agents have been demonstrated to have a promising therapeutic influence on various neurodegenerative disorders associated with diabetes and oxidative stress. *Eucalyptus globulus* (Fever tree) belongs to the family of Myrtaceae (Akin *et al*., 2012). It is the commonest eucalyptus (Damjanović-Vratnica *et al*., 2011), and grows in a wide range of conditions. It is used as a traditional treatment for diabetes and previous studies showed its anti-inflammatory, antioxidant and antidiabetic properties (Gray & Flatt, 1998). Therefore, this study was designed to investigate the neuropharmacological effect of ethanol leaf extract of *Eucalyptus globulus* in diabetes mellitus with a focus on its effect on brain redox status and disturbed neurochemistry.

MATERIALS AND METHODS

Chemicals and Reagents: Thiobarbituric acid (TBA), trichloroacetic acid (TCA), reduced glutathione (GSH), glutamic acid, adenosine triphosphate (ATP), 6,7-Dimethyl-5,6,7,8-tetrahydropterine (DMTHP), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), perchloric acid (PCA), benzylamine hydrochloride (BAHC), dopamine hydrochloride, reduced nicotinamide-dinucleotide(NADH), ethylenediaminetetraacetic acid (EDTA), 1-amino-2-naphthol-4-sulphonic acid (ANSA), 2,4-dinitrophenyl hydrazine (DNPH), acetylcholine iodide, epinephrine, tetramethylbenzidine (TMB) streptozotocin and ammonium molybdate were obtained from Sigma-Aldrich (St-Louis, MO, USA). Glibenclamide was obtained from HOVID Bhd. (Darul Ridzuan, Malaysia). All other chemicals and reagents were of analytical grade and obtained from standard sources.

Plant material and preparation of extract: The leaves of *Eucalyptus globulus* were collected from a fully grown tree at Oda (7◦12′40.4″N 5◦12′45.7″E) in Akure, Nigeria in August 2018. The leaf was authenticated at the Herbarium of the Federal University of Technology, Akure, Nigeria where a voucher sample (voucher number: 0265) was deposited.

The leaves were air-dried at room temperature and pulverized using an electric grinder. The technique of continuous hot extraction using a Soxhlet extractor was employed. The ethanol leaf extract was prepared by refluxing 200 g of the plant sample with 1000 ml of absolute ethanol for 12 h. The extract obtained was stored in a refrigerator for further use. The yield of the extract (EG) was 35.59 g. EG was standardized by HPLC-DAD fingerprinting.

Quantification of compounds in EG by HPLC-DAD fingerprinting: The extract (10 mg) was dissolved in aqueous acetonitrile (10 mg/20 ml) and mixed vigorously for 30 min. After mixing, the aqueous end was run off while the organic solvent end was collected into a 25 ml standard flask. The analysis was performed on a Shimadzu (NexeraMX) HPLC system fitted with a uBONDAPAK C18 column (length 100 mm, diameter 4.6 mm, and thickness 7 μm). The mobile phase consisted of a mixture of aqueous acetonitrile (acetonitrile/water, 80:20). The sample was injected at a volume of 5 μl and the flow rate was set at 0.08 ml/min for water and 5 ml/min for acetonitrile at a pressure of 15 mpa. Compounds were detected by a UV detector at 254 nm (Diode Array Detector, DAD). The retention times of the identified compounds of interest were measured using a standard solution at a concentration of 15.69 mg/g. The extract was injected into the high-performance liquid chromatographic machine to obtain a curve providing peak area and retention time in a chromatogram. Then the peak area of the sample is compared with that of the standard relative to the concentration of the standard to obtain the concentration of the sample and their concentration was calculated as shown below:

Concentration=

$$\frac{peak\ area\ of\ compound \times standard\ concentration}{peak\ area\ of\ standard}$$

Induction of type-2 diabetes mellitus: Healthy male Wistar rats at 10 weeks of age and weighing 140–200 g were used for these studies. Rats were fed chow (15% crude protein, 4% crude fat, 6% crude fiber, 1.1% calcium, 0.3% phosphorus, 0.7% lysine, 0.25% methionine) and water, ad libitum. The animals were acclimatized for 7 days before studies began. The experiments were approved by the animal research ethics committee of the Federal University of Technology, Akure, Nigeria (FUTA/ETH/21/05). Animal research was conducted following the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Rats were provided with 10% fructose in drinking water for 2 weeks after which a single dose of 40 mg/kg STZ was administered by intraperitoneal injection (Obafemi *et al*., 2019). After 72 h of STZ injection, blood was collected from the tail vein for blood glucose level determination. Animals with fasting blood glucose levels > 250 mg/dl were considered diabetic and used for the experiment. Rats were randomly divided into ten groups with nine animals per group as follows: normal control, diabetic control, diabetic + 100 mg/kg EG, diabetic + 200 mg/kg EG, diabetic + 400 mg/kg EG, diabetic + 5 mg/kg glibenclamide, 100 mg/kg EG, 200 mg/kg EG and 400 mg/kg EG. The extract was dissolved in distilled water. A uniform suspension of the standard drug glibenclamide in distilled water was prepared. Both extract and reference drug were administered by oral gavage. The normal control and diabetic control groups received distilled water. Treatment lasted for 21 days after which the animals were fasted overnight before sacrifice. After sacrifice, three animals were randomly picked for histopathological evaluation and gene expression analysis.

Biochemical estimations: The brains of the sacrificed rats were excised and washed in ice-cold 1.15% (w/v) potassium chloride solution, blotted with filter paper, and weighed. They were then blended in 0.1 M, pH 7.4 phosphate-buffered saline (PBS) using a Teflon homogenizer, to prepare a 10% (w/v) homogenate which was centrifuged at 10,000 x g at 4 °C for 25 min to obtain the supernatant used for biochemical analyses. Blood was collected through cardiac puncture and stored in sample tubes. It was later

centrifuged and the serum was collected and used for biochemical estimations.

Estimation of glucose and electrolytes: Glucose, potassium ion, and sodium ion concentrations were estimated in the serum using assay kits obtained from Randox Laboratories Ltd (Crumlin, UK) according to instructions provided by the manufacturer.

Determination of hematological indices: Hematocrit was determined using high-speed centrifugation of blood-filled hematocrit tubes with a Zipocrit Hematocrit Centrifuge (ThermoFisher Scientific, Philadelphia, PA). All white blood cell (WBC) count estimates were performed by the same technician, at a location on the slide where the cells were one layer thick, adjacent to one another (membranes touching), evenly distributed, and showed no signs of morphological change. White blood cell estimates were made by using a 100X objective lens with immersion oil, counting the number of white blood cells in 10 fields, calculating the average, and then multiplying the number of cells by 2000. The absolute cell count for each type of cell was calculated by multiplying the percentage of the type of cell by the overall WBC estimate.

Assessment of brain redox indices: Reduced glutathione concentration was estimated using the method of Beutler (Beutler *et al*., 1963), and the assessment of lipid peroxidation was carried out using the method described by Varshney and Kale (Varshney & Kale, 1990). Superoxide dismutase activity was measured using the method of Misra and Fridovich (Misra & Fridovich, 1972) while Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation and oxidative stress was evaluated according to the method of Eiserich and others (Eiserich *et al*., 1998).

Assessment of markers of neurochemical disturbances: The effect of diabetes and EG treatment on brain Na+ K+ ATPase activity was evaluated as previously described (Svoboda & Mosinger, 1981). Acetylcholinesterase (AChE) activity in brain homogenate was measured as previously described (Ellman *et al*., 1961). The turnover of the striatal catecholamine, dopamine, was estimated by measuring the activity of tyrosine hydroxylase (Shiman *et al*., 1971), the level of dopamine (Guo *et al*., 2009), and the activity of MAO (Holt *et al*., 1997) using previously established methods.

Statistical analysis: Results were expressed as mean ± standard deviation (SD). All data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. Values of $p < 0.05$ were considered statistically significant. All the statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The phytoconstituents and their respective concentrations in the extract as revealed by HPLC-DAD fingerprinting in Table 1 show that ergosterol (10.55 ± 0.15 mg/g) and catechin (10.05 ± 0.10 mg/g) were the most abundant phytochemicals in the plant extract. Pinitol and quercetin were also detected.

Table 1:
HPLC-DAD quantified compounds of *Eucalyptus globulus* ethanol leaf extract

Compounds	Concentration (mg/g)
Catechin	10.05±0.10
Catechol	2.19±0.04
Catecholamine	2.71±0.03
Catechutannic acid	0.20±0.01
Quercetin	2.41±0.13
Ergosterol	10.55±0.15
Apigenin	0.70±0.02
Luteolin	0.29±0.01
Robinetinidol	0.24±0.08
Pinitol	0.18±0.001

Results are expressed as mean ± standard deviations of two determinations.

Table 2:
Effect of *Eucalyptus globulus* leaf extract on weight change and serum glucose of fructose/streptozotocin-induced diabetic rats

Group	Weight Gain/ Loss (g)	Glucose concentration (mg/dl)
Negative control	4.00±1.66	43.17±11.17
Diabetic control	-28.43±7.8$^{\#\#\#\#}$	258.30±2.28$^{\#\#\#\#}$
Diabetic + 100EG	11.8±2.21****	150.93±17.21****
Diabetic + 200EG	13.34±3.54****	133.05±4.99****
Diabetic + 400EG	16.86±5.66****	103.24±7.95****
Diabetic + GLI	-2.66±0.15****	94.92±9.39****
100EG	2.00±0.24****	42.74±6.81****
200EG	4.00±0.33****	38.42±5.02****
400EG	7.67±1.67****	34.75±7.81****

Results are expressed as Mean ± SD (n=6). $^{\#\#\#\#}P<0.0001$ vs negative control; $^{****}P<0.0001$ vs diabetic control. EG: ethanol leaf extract of Eucalyptus globulus , GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract.

Table 3:
Effect of EG on serum sodium and potassium ion levels of diabetic rats

GROUP	K^+ (mEq/l)	Na^+ (mEq/l)
Negative control	6.05±0.15	121.54±0.64
Diabetic control	1.89±0.14$^{\#\#\#\#}$	80.12±2.35$^{\#\#\#\#}$
Diabetic + 100EG	3.53±0.12****	93.43±0.64****
Diabetic + 200EG	3.75±0.20****	96.43±0.43****
Diabetic + 400EG	4.22±0.38****	102.10±0.64****
Diabetic + GLI	4.55±0.25****	100.82±0.21****
100EG	6.02±0.09****	120.86±0.21****
200EG	6.00±0.06****	117.08±0.43****
400EG	6.10±0.08****	123.35±0.43****

Results are expressed as Mean ± SD (n=6). $^{\#\#\#\#}P<0.0001$ versus negative control; $^{****}P<0.0001$ versus diabetic control; EG: ethanol leaf extract of Eucalyptus globulus , GLI: Glibenclamide, 100EG: 100 mg/kg Eucalyptus globulus ethanol leaf extract, 200EG: 200 mg/kg Eucalyptus globulus ethanol leaf extract, 400EG: 400 mg/kg Eucalyptus globulus ethanol leaf extract, K^+: potassium ion and Na^+: sodium ion.

Table 4:
Effect of *Eucalyptus globulus* on the hematological indices of fructose/streptozotocin-induced diabetic rats

GROUP	PCV %	HB g/dl	RBC1x0^9/L	WBC x 10^9/L	Neu %	Lym %
Negative control	42.33 ± 2.12	14.43±0.56	4.47±0.35	6700 ± 494	22.33 ± 0.71	74.67 ± 2.83
Diabetic control	27.00 ± 1.41$^{\#\#\#\#}$	11.4±0.21$^{\#\#\#\#}$	2.63±0.09$^{\#\#\#\#}$	4233 ± 251$^{\#\#\#\#}$	35.33 ± 1.41$^{\#\#\#\#}$	62.33 ± 2.82$^{\#\#\#\#}$
Diabetic + 100EG	35.67±0.70****	12.87±0.07***	3.59±0.32**	4433 ± 70	28.33 ± 0.71****	69.33 ± 1.15****
Diabetic + 200EG	37.00±0.70****	13.56±0.07****	3.72±0.21***	4933 ± 115	26.00 ± 1.41****	71.33 ± 1.52****
Diabetic + 400EG	38.67±2.12****	13.96±0.28****	4.11±0.24****	5700 ± 353****	25.33 ± 2.82****	72.67 ± 2.12****
Diabetic + GLI	34.00±0.00****	12.03±0.35****	3.47±0.08**	5900 ± 251****	22.67 ± 2.82****	73.00 ± 2.12****
100EG	35.67±0.70****	12.87±0.07***	3.59±0.32**	6733 ± 494****	21.67 ± 0.71****	76.33 ± 1.52****
200EG	42.00±2.12****	14.83±0.29****	4.33±0.15****	6766 ± 636****	21.33 ± 0.71****	75.33 ± 1.41****
400EG	43.67±2.12****	14.9±0.07****	4.56±0.21****	6833 ± 353****	20.67 ± 1.41****	75.67 ± 1.15****

Results are expressed as Mean ± SD (n=6). $^{\#\#\#\#}$P<0.0001 versus negative control; **P<0.01, ***P<0.001, ****P<0.0001 versus diabetic control; EG: ethanol leaf extract of *Eucalyptus globulus* , GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract, PCV: packed count volume, HB: hemoglobin, RBC: red blood cell, WBC: white blood cell count, NEU: neutrophils and LYM: lymphocytes.

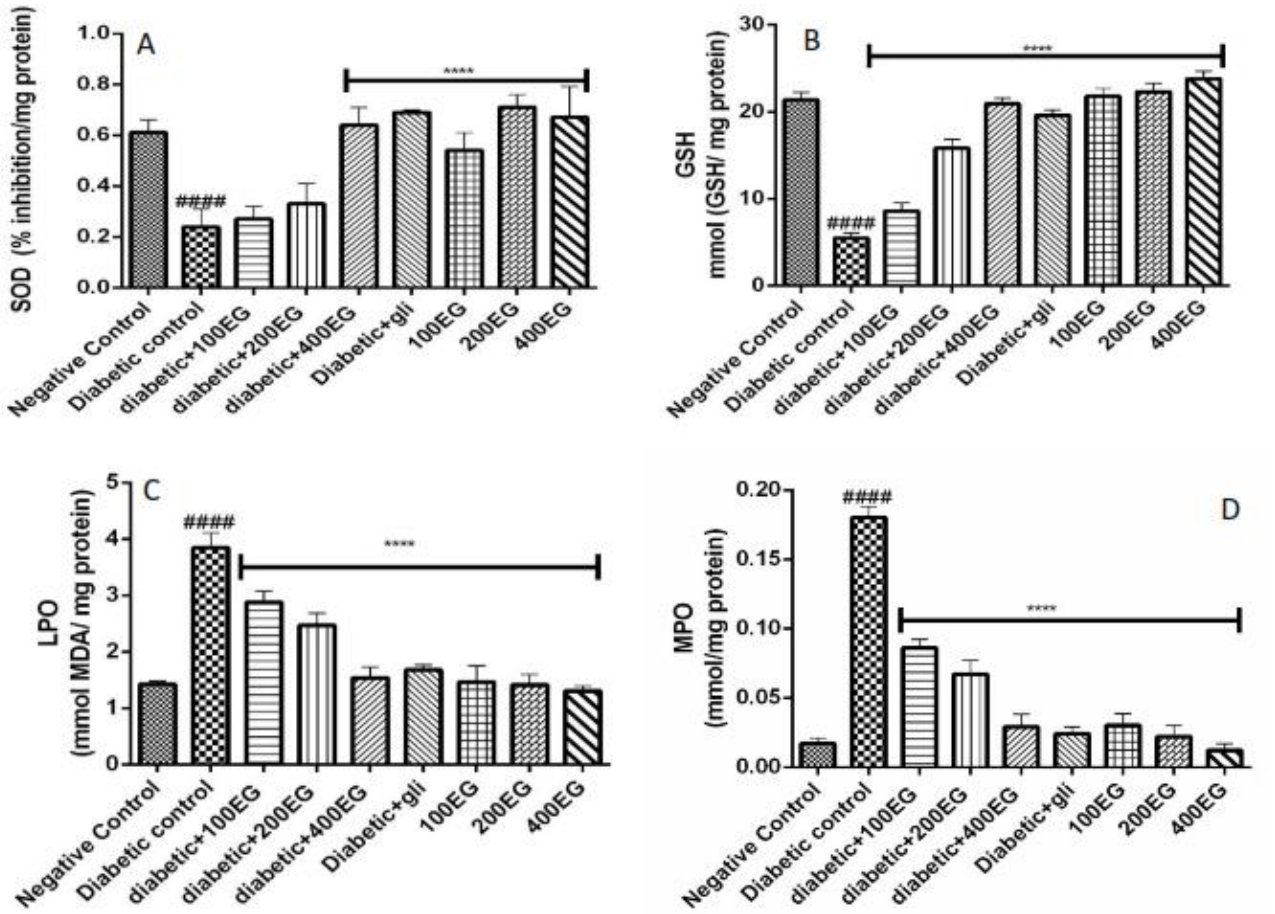

Figure 1:
Effect of *Eucalyptus globulus* on redox stress in the brain tissue of fructose/streptozotocin-induced diabetic rats. (A) superoxide dismutase activity, (B) reduced glutathione concentration, (C) extent of lipid peroxidation and (D) myeloperoxidase activity. Each bar is expressed as mean ± standard deviation (n=6). ####P<0.0001 vs. negative control; ****P<0.0001 vs. diabetic control; GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract, SOD: superoxide dismutase, GSH: reduced glutathione, LPO: lipid peroxidation, MDA: malondialdehyde, MPO: myeloperoxidase.

The induction of diabetes caused a significant ($p < 0.0001$) decrease in the weight of the animals while EG treatment resulted in a significant ($p < 0.0001$) gain in weight. EG at 400 mg/kg produced a 12% weight gain but treatment with glibenclamide did not correct the loss in weight. EG administration to diabetic rats significantly ($p < 0.0001$) ameliorated the induced hyperglycemia (Table 2). The groups administered glibenclamide had the least serum glucose level at the end of the experiment which was not significantly different from the glucose level of animals administered 400 mg/kg EG.

Hyponatremia and hypokalemia were observed in the untreated diabetic group as shown in Table 3. Treatment with the extract and glibenclamide ameliorated the electrolyte disturbances. The effect of the ethanolic extract of E. globulus on the hematological parameters of normal

and diabetic rats is presented in Table 4. Untreated diabetic rats showed a significant decrease ($p<0.0001$) in packed cell volume, red blood cells, white blood cells, hemoglobin, and lymphocytes but increased neutrophils when compared with the normal control group. Administration of ethanolic extract of E. globulus ameliorated these changes in hematological indices.

Figure 1 shows that induction of diabetes led to brain redox imbalance as revealed by the decreased SOD activity (Figure 1A) and GSH level (Figure 1B), and the increased lipid peroxidation (Figure 1C) and myeloperoxidase activity (Figure 1D). This redox imbalance was ablated by treatment with EG. The 400 mg/kg extract-treated group showed the highest anti-oxidative stress activity.

As shown in Figure 2, altered brain neurochemistry was reflected in decreased Na+K+ ATPase activity (Figure 2A), increased acetylcholinesterase activity (Figure 2B), decreased tyrosine hydroxylase activity (Figure 2C), decreased dopamine level (Figure 2D), and increased monoamine oxidase activity (Figure 2E) in the brain of diabetic control animals compared with the normal control. These alterations were ameliorated in diabetic animals administered EG with the 400 mg/kg dose presenting the best activity.

DISCUSSION

Poor control of hyperglycemia is associated with the development and progression of complications such as brain dysfunction in diabetic patients. Progressive cognitive deterioration, low intelligent quotient, neurodegeneration, dementia, and brain atrophy are frequent consequences of diabetes mellitus (Hamed, 2017). Mechanisms of brain injury in diabetes mellitus include inflammation, increased acetylcholinesterase (AChE) activity, defective neurotrophic factors, neurotransmitter changes, and other vascular risk factors. Decreased level of acetylcholine was reported to contribute to cognitive deficits observed in diabetic rats (Maciel *et al*., 2016). Results from the present study support these observations.

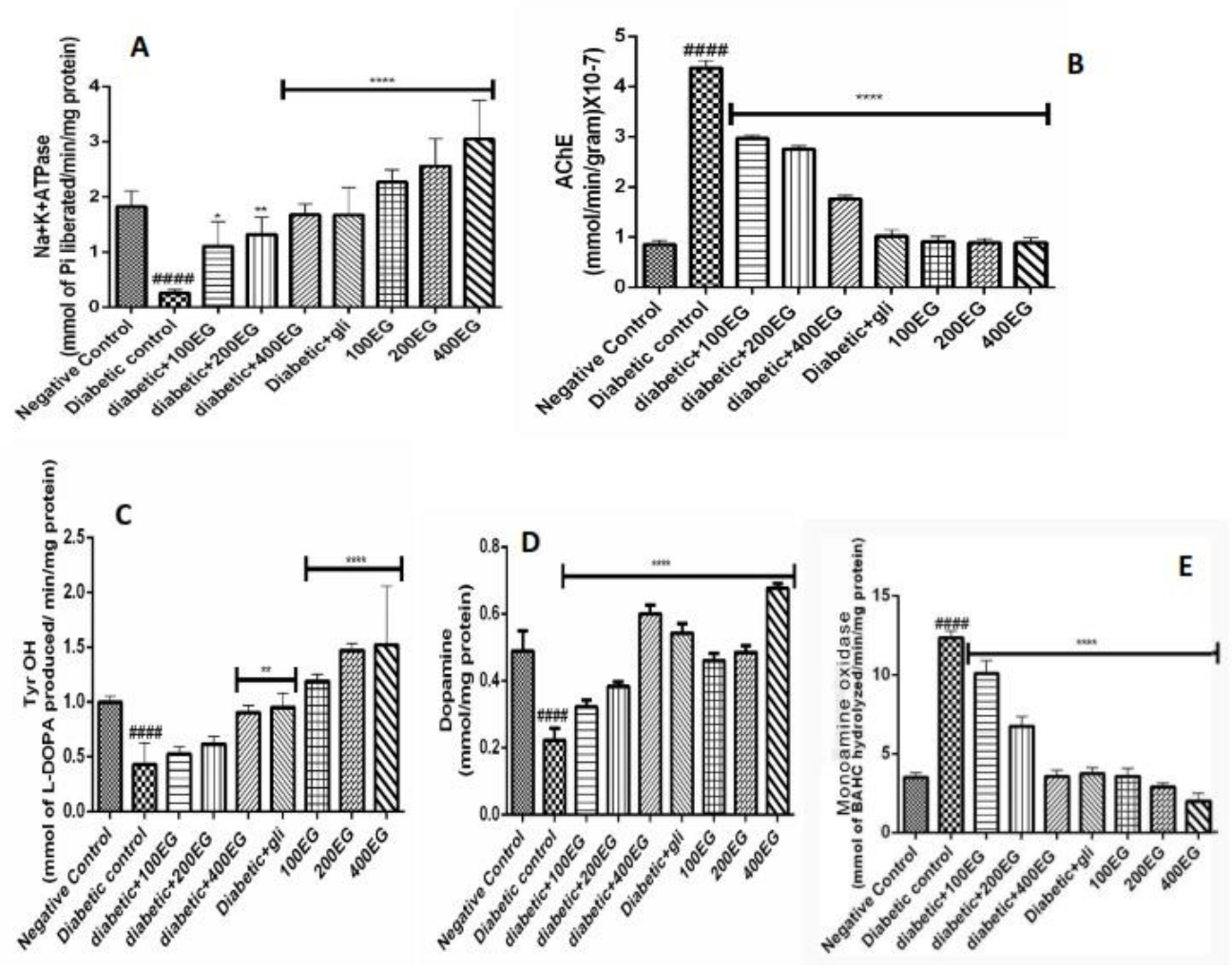

Figure 2:
Effect of *Eucalyptus globulus* on altered brain chemistry of fructose/streptozotocin-induced diabetic rats. (A) Na^+K^+ATPase activity, (B) acetylcholinesterase activity, (C) tyrosine hydroxylase activity, (D) dopamine level, and (E) monoamine oxidase activity. Each bar is expressed as mean ± standard deviation (n=6). $^{####}P<0.0001$ vs negative control; $^{****}P<0.0001$ vs. diabetic control; GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract, Na^+P^+ATPase: sodium-potassium adenosine triphosphatase, AChE: acetylcholinesterase, Tyr OH: Tyrosine hydroxylase.

Some of the detected phytoconstituents in the extract have been shown to have antidiabetic potential. Ergosterol may be a potential hypoglycemic agent for the treatment of type-2 diabetes mellitus with the probable mechanism of stimulating GLUT4 translocation and expression. Catechin, a flavonoid polyphenol has been reported to possess antidiabetic property (Mrabti *et al*., 2018). The neuroprotective effect of catechin has also been documented (Cheruku *et al*., 2018; Jiang *et al*., 2017). Pinitol and quercetin have been reported to possess antidiabetic effects. D-Pinitol showed an antihyperlipidemic effect in STZ-induced type-2 diabetes mellitus (Geethan & Prince, 2008) and prevented lipid peroxidation by increasing cellular antioxidant levels (Rengarajan *et al*., 2015) while quercetin was reported to lower serum glucose at a dose as low as 10 mg/kg (Bule *et al*., 2019). The presence of Pinitol, Ergosterol, and Catechin in the E. globulus leaf extract could have contributed to the observed antidiabetic and neuroprotective effects.

EG administration to diabetic rats ameliorated the induced hyperglycemia. Of note is the observation that the effectiveness of the extract at 400 mg/kg was comparable to that of glibenclamide, the reference standard drug. Also, the improvement of weight abnormalities of diabetic rats by EG supports its antidiabetic activity. Weight changes may accompany diabetes mellitus as observed in the present study. This may result from aberrations in lipid and protein catabolism.

Treatment with the extract and glibenclamide ameliorated the electrolyte disturbances. Electrolyte disturbances are features of diabetes and are also associated with brain dysfunction. Electrolytes are involved in controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting, and muscle contraction. Potassium, sodium, and calcium are all important for proper electrolyte balance (Khanduker *et al*., 2017). In this study, the induction of diabetes caused an abnormally low level of potassium ions and sodium ions in the serum of diabetic rats. Hyponatremia is the most common electrolyte abnormality in clinical practice and is associated with increased morbidity and mortality (Waikar *et al*., 2009). Imbalance in sodium level is associated with an increased probability of adverse outcomes such as cognitive impairment, osteoporosis, and fracture (Podestà *et al*., 2015). Hypokalemia is associated with impaired insulin secretion and decreased peripheral glucose utilization resulting in carbohydrate intolerance and hyperglycemia (Khanduker *et al*., 2017). Amelioration of these conditions by EG treatment indicates its antidiabetic and neuroprotective effects.

Untreated diabetic rats showed alterations in hematological indices which were ameliorated by the administration of EG. White blood cell and platelet abnormalities are common among people with diabetes. The reduction in the total white blood cell count of the diabetic control group indicated a weak immunity against infection and diseases. Neutrophils participate in both protective and detrimental responses to a diverse array of inflammations and infections (Witter *et al*., 2016). Chronic inflammation in adipose tissue is considered a crucial risk factor for the development of insulin resistance and type-2 diabetes in obese individuals (Zatterale *et al*., 2020). An increase in the neutrophil count of the diabetic control group in this study is an indication of inflammation which was ameliorated by treatment with EG. Anaemia is a common phenomenon in diabetes and the main factor contributing to its prevalence in diabetes is the inability of the kidney to increase erythropoietin secretion in response to decreased hemoglobin (Obafemi *et al*., 2017). The occurrence of anemia in diabetic conditions has been established (Barbieri *et al*., 2015) and this is in agreement with the report of this study. Anemia or low hematocrit has been associated with impaired brain function, neurological injury, and increased mortality, indicating that the brain is vulnerable to anemia-induced injury (Hare, 2004). The increase in packed cell volume, red blood cell count, and hemoglobin level in all the EG-treated groups suggests the neuroprotective effect of EG in diabetic rats.

The induction of diabetes led to brain redox imbalance which was ablated by treatment with EG. The 400 mg/kg extract-treated group showed a protective effect. Myeloperoxidase (MPO) is a mammalian pro-oxidant and pro-inflammatory enzyme mainly released by activated neutrophils that have been implicated in the progression of diabetes (Adedara*et al*., 2019a). The observed decrease in the activity of MPO in the brain tissue after treatment with EG along with the observed decrease in neutrophil counts in all EG treated groups supports the anti-inflammatory and antioxidative activities of the extract.

The altered brain neurochemistry of diabetic control animals was ameliorated in diabetic animals administered EG. Altered function of the electrogenic transmembrane ATPase, Na+K+ ATPase, is common in brain dysfunctions (Ojo *et al*., 2019; Pop-Busui *et al*., 2017). Sodium pump dysfunction may lead to neuronal depolarization, release of excess neurotransmitters (such as glutamate), and neuronal excitotoxicity. The reduction in the activity of Na+K+ ATPase observed in untreated diabetic rats and the increase in the activity of the enzymes in EG-treated animals support the neuroprotective potential of the extract. Furthermore, disturbed neurotransmitter metabolism in diabetic conditions and its various consequences on neurological functions have been documented (Parashar *et al*., 2018; Prabhakar *et al*., 2015). Dopamine functions as a neurotransmitter with regulatory functions in movement, memory, pleasurable reward, behavior and cognition, and attention. The direct precursor of dopamine, L-DOPA, is generated via the action of tyrosine hydroxylase that catalyzes the conversion of L-Tyrosine to L-DOPA. Monoamine oxidase on the other hand is involved in the catabolism of dopamine. Acetylcholinesterase is a neurotransmitter metabolizing enzyme. It catalyzes the breakdown of acetylcholine during the process of neurotransmission. The observed increase in the activity of acetylcholinesterase in diabetic rats in this present study corroborates earlier findings (Adedara *et al*., 2019a, 2019b; Rajput & Sarkar, 2017). Both dopaminergic and acetylcholinergic neurotransmission critically modulate synaptic transmission, plasticity, and motor coordination (Ojo *et al*., 2019). Treatment with EG reduced the activity of acetylcholinesterase thereby making more acetylcholine available in the synapses for neurotransmission. EG treatment also increased the production of dopamine via optimization of tyrosine hydroxylase activity with simultaneous suppression of monoamine oxidase activity. The attenuation of diabetes-induced neurotransmitter

dysregulation by EG treatment further shows its beneficial property in diabetic brain dysfunction.

In conclusion, this study has revealed the neuropharmacological property of *Eucalyptus globulus* in a model of type-2 diabetes. Results from this research revealed the antidiabetic property of the extract and its neuroprotective property against diabetes-induced brain redox stress and altered neurochemistry. The plant is therefore of potential therapeutic relevance in managing diabetic complications, especially diabetic brain injury. Further studies on the neuroprotective effect of *Eucalyptus globulus* phytoconstituents are recommended to give insights into the roles and contributions of the compounds identified in the extracts.

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Full length Research Article

Phoenix dactylifera and Polyphenols Ameliorated Monosodium Glutamate toxicity in the Dentate Gyrus of Wistar Rats

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Summary: Monosodium glutamate (MSG) has been known to cause neurodegeneration, due to its ability to trigger excitotoxicity, and the hippocampus is one of the most affected regions. Therefore, *Phoenix dactylifera* (*P. dactyliferaa*) and polyphenols were employed in this study to mitigate on the deleterious effect of monosodium glutamate on the dentate gyrus of Wistar rats. Forty-eight male Wistar rats weighing between 120-150g was used for the study. The Wistar rats were grouped into eight, (n=6). Groups 1-8 received 1.6mL/kg normal saline, 4000mg\kg monosodium glutamate for 7-days, 4000mg\kg monosodium glutamate for 7-days and 100mg\kg caffeic-acid for 14-days concurrently, 4000mg\kg monosodium glutamate for 7-days and 100mg\kg *P. dactyliferaa* for 14-days concurrently, 4000mg\kg monosodium glutamate for 7-days and 100mg\kg luteolin for 14-days concurrently, 100mg\kg. caffeic-acid for 14-days followed by 4000mg\kg monosodium glutamate for 7-days, 100mg\kg *P. dactyliferaa* for 14-days followed by 4000mg\kg monosodium glutamate for 7-days and 100mg\kg luteolin for 14-days followed by 4000mg\kg monosodium glutamate for 7-days respectively. After the treatments, the rats underwent behavioural test (Y-maze test), and subsequently, the brain tissues were processed for histological (Hematoxylin & Eosin stain) and biochemical (superoxide dismustase, glutathione peroxidase and malondialdehyde) analyses. The activities of *P. dactyliferaa* and polyphenols ameliorated the deleterious effect of monosodium glutamate, through increased spontaneous alternation of the experimental animals, dominant matured granule cells of the dentate gyrus and modulated the activities of superoxide dismutase, glutathione peroxidase and malondialdehyde in the male Wistar rats. Therefore, this study revealed that *P. dactyliferaa* and polyphenols ameliorated monosodium glutamate toxicity in the dentate gyrus of Wistar rats.

Keywords*: Glutamate toxicity, Dentate gyrus, Phoenix dactylifera, Oxidative stress, Neurodegeneration*

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INTRODUCTION

Glutamate is the main constituent of dietary protein and is also consumed in many foods as additive and flavourings in homes and restaurants, in the form of monosodium glutamate (Airaodion *et al*., 2019). It is made of a nutritionally indispensable amino acid, glutamic acid (Zehra *et al*., 2017). Despite its deleterious effect, it remains the major food addictive in most low-income countries. Glutamate is the excitatory neurotransmitter in the mammalian Central Nervous System (CNS) playing an important role in both physiological and pathological processes (Airaodion *et al*., 2019). Receptors of glutamate are dispersed throughout the CNS especially in the amygdala, hippocampus and hypothalamus where they regulate many vital metabolic and autonomic functions. Monosodium glutamate (MSG) is a sodium salt of glutamic acid and has almost same structure with glutamate, the difference is that one hydrogen atom at the carboxylic chain has been replaced with a sodium atom, hence, the name (Airaodion *et al*., 2019). Monosodium glutamate has a meaty taste and due to this, many food producers use MSG to enhance the flavour of their products. Monosodium glutamate could cause neurodegeneration due to its ability to trigger excitotoxicity when consumed in high amount, and the hippocampus is one of the most affected regions, the granule cells of the dentate gyrus being its major input area (Abdel *et al*., 2018).

Neuronal dysfunction and death is a complex phenomenon that involves failure of metabolic processes, protein mitochondrial dysfunction, increased oxidative stress, defective proteasome system, protein aggregation, changes in iron metabolism, and excitotoxicity and inflammation (Dajas *et al*., 2013). The process of MSG triggering excitotoxicity plays a major role in the initiation, as well as the progression of neurocognitive and locomotor disorders (Ahmed *et al*., 2015; Renaud *et al*., 2015). Therefore, it is imperative to stop the progression of MSG insult in the brain.

Consumption of polyphenolic rich diets helps reduce the risk of chronic human diseases. Phenolic groups in polyphenols have the ability to accept an electron to form relatively stable phenoxyl radicals, phenoxyl radical in turn disrupts chain oxidation reactions in cellular components (Pandey and Rizvi, 2009). Polyphenols can be classified

into flavonoid and non-flavonoid polyphenolic compounds. Luteolin is a form of flavonoid while caffeic acid is a form of non-flavonoid polyphenolic compouind. *Phoenix dactylifera* is said to be packed with lots of polyphenolic compound. Luteolin, caffeic acid and *P. dactyliferaa* have been used in this study against neurotoxicity. Furthermore, it has been reported that polyphenol-rich foods and beverages may increase plasma antioxidant capacity (Tsao 2010). Also, Polyphenols has been suggested to protect cell constituents against oxidative damage, thereby limiting the risk of various degenerative diseases associated with oxidative stress (Luqman and Rizvi, 2006; Pandey *et al*., 2009; Pandey and Rizvi, 2009). *Phoenix dactylifera* (*P. dactyliferaa*) which belongs to the family Arecaecae (Sami *et al*., 2017) is referred to as date palm fruit in English, 'dabino' by the Hausas while the Yorubas call it 'labidun' (Mustafa *et al*., 1986; Biglari *et al*., 2008). The extensive nutraceutical values of *P. dactyliferaa* have been documented to include anticancer, antimutagenic, antioxidant and anti-inflammatory effects among others (Vyawahare *et al*., 2009; Pujari *et al*., 2014). *Phoenix dactylifera* fruit extract contains various phenolic compounds such as caffeic acid (dactyliferic acid), ferulic acid, luteolin, and quercetin. (Allaith, 2005) implicated for the nutraceutic activities above, but the antioxidant activity of *P. dactyliferaa* is majorly related to the total phenolic contents (Biglari *et al*., 2008). Caffeic acid (3, 4-dihydroxycinnamic acid), an important constituent of *P. dactyliferaa* , is a non-flavanoid catecholic compound present in other plants (Clifford, 1999), with a broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant and immunomodulatory effects (Chan and Ho, 1997; Gulcin, 2006). It has been established that phenolic acids bearing a carbonyl group separated from the aromatic ring (e. g. cinnamic acid, caffeic acid) are able to exhibit more potent pharmacological properties than their counterparts where the carbonyl is directly linked to the aromatic ring (Luc *et al*., 2012). Luteolin (3, 4, 5, 7-tetrahydroxy flavone), another important flavanoid in *P. dactyliferaa* , is naturally found in several other plant species (Kim *et al*.,2000; Lopez-Lazaro, 2009), also has antioxidant, anticancer, anti-inflammatory, and neuroprotective properties (Chen *et al*., 2008). Presence of four hydroxyl moieties, oxygen molecules, and carbon-carbon double bonds in luteolin is advantageous to the various pharmacological properties its able to exhibit (Kim *et al*., 2000; Nabavi *et al*., 2009).

Numerous studies have asserted that MSG causes neurotoxicity, oxidative stress, inflammation and predisposition and worsening of the neurological disorders. However, there is persistent increase in MSG consumptions, thus contributing to the growing population of persons living with neurodegenerative diseases (Eweka, 2007; Hughes, 2009). Although there are potent glutamate-releasing inhibitors (glutamatergic drugs), but with several side effects, thus the need for novel natural agents with possible therapeutic effects in MSG neurotoxicity, which *P. dactyliferaa*, caffeic acid and luteolin are potential candidates.

Hence, the study aimed at investigating the ameliorative effect of luteolin, caffeic acid as well as *P. dactyliferaa* on MSG-induced neurotoxicity in the dentate gyrus of Wistar rats. Therefore, it is imperative to find affordable means of combating MSG toxicity.

MATERIALS AND METHODS

Chemicals: Luteolin (CAS No 491-70-3) and caffeic acid (CAS No 331-39-5) were obtained from Henan Kaixiang Biological Technology Ltd. China. Dried fruits of *Phoenix dactylifera* were obtained at Sango area of Ilorin, Kwara State and were certified at the Department of Plant Biology at the University of Ilorin, with voucher number UILH/001/1205. Analytical grade of methanol (CAS 67-56-1) was procured from Central Research Laboratory, Tanke, Ilorin for the methanolic crude extract of *Phoenix dactylifera* fruits.

Experimental Animals: Forty-eight male Wistar rats (120-150 g) were used for this study. These animals were accommodated in the animal holdings of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, where they had free access to food and water in accordance to the Guide for the Care and Use of Laboratory Animals of University of Ilorin, Ilorin Kwara State. Ethical approval was obtained from the Ethical Committee of the University of Ilorin through the Faculty of Basic Medical Sciences, University of Ilorin (UERC\ASN\2018\1258).

Experimental Design: The animals were grouped into eight, with six animals in each group (n=6). Groups 1-8 received the following treatment respectively; oral administration of 1.6mL/kg normal saline; 4000mg\kg monosodium glutamate (Faronmbi and Onyema, 2006) for 7 days; 4000mg\kg monosodium glutamate for 7 days and 100 mg\kg caffeic acid (Anwar *et al*., 2012) for 14 days concurrently; 4000mg\kg monosodium glutamate for 7 days and 500 mg\kg *Phoenix dactylifera* (Dibal *et al*., 2016) for 14 days concurrently; 4000mg\kg monosodium glutamate for 7 days and 100 mg\kg luteolin (Lu *et al*., 2015) for 14 days concurrently; 100 mg\kg.caffeic acid for 14 days followed by 4000mg\kg monosodium glutamate for 7 days; 500 mg\kg *Phoenix dactylifera* for 14 days followed by 4000mg\kg monosodium glutamate for 7 days; and 100 mg\kg luteolin for 14 days followed by 4000mg\kg monosodium glutamate for 7 days respectively.

Behavioural Analysis: The memory index of the animals was assessed with the Y maze test. The Y maze test was majorly used to assess spatial working memory in experimental rats. The test was conducted on day 21 of administration in a Y-shaped maze with opaque wooden arms. The three arms were of equal length (30 cm), width (10 cm) and height (15 cm). The arms were interconnected at a 120° angle from each other. The experimental animals were placed in the centre of the maze, (i.e. the junction that connects the 3 arms) one after the other. The animals were allowed to explore the three arms freely for 5 minutes. The number of arm entries and the number of triads was recorded; this was used to calculate the percentage alternation. It is important to note that an entry was being recorded when all four paws of the experimental animals were within the arm. The maze was cleaned with 95% ethanol in between two trials. This was to ensure that they

did not trace the odour of the previous animal, which could cause bias in their trend of movement. However, the percentage alternation was calculated using the formula stated below.

Percentage alternation=

$$\frac{number\ of\ complete\ triad\ enteries}{number\ of\ arm\ enteries - 2} x\ 100$$

(Hughes, 2004; Oriel and Kofman, 2015).

Animal Euthanization: After completion of administration, the rats were euthanized using ketamine on day 22. Two animals were perfused through the heart with 4% paraformaldehyde and their brain tissues excised. To obtain a coronal section of the hippocampus, the whole brain was cut coronally into two halves at the highest point and sections were taken 2mm away from the both halves. The tissue block produced was sectioned with the aid of a rotary microtome. The tissue sections were at 5 microns thick while the knife was placed at 45° to the block of wax containing the tissue. The other animals were not perfused and their brain tissues were homogenized for biochemical assays.

Histological Analysis: The haematoxylin and eosin (H and E) (Sheehan and Hrapchak 1987) staining technique was used to demonstrate the general histo-architecture of the cells; to show location of normal or abnormal nucleus of the granule cells in the granule layer of the dentate gyrus.

Determination of Biochemical Parameters: The 0.1g of the hippocampus was homogenized in 0.4 ml of 5% sucrose solution and taken to the centrifuge. The homogenate was spun for 10 minutes at 5000 revolutions per minute and the supernatants were placed in plain bottles and taken for analysis of superoxide dismutase, glutathione peroxidase and malondialdehyde. The estimation of superoxide dismutase was carried out using the methods of Sun and Zigma (1978), malondialdehyde was estimated using the methods of Buege and Aust (1978) and glutathione peroxidase was estimated using the methods of Reddy *et al*. (1995).

Statistical Analysis: Values were reported as means ± standard error of mean (SEM), comparison amongst groups was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison and considered significant at $p < 0.05$. Graph pad prism version 7.0 was used for the statistical analysis.

RESULTS

Spontaneous Alternation Test: The percentage spontaneous alternation in the Y maze by the Wistar rats after exposure to normal saline, MSG, caffeic acid, luteolin and *Phoenix dactylifera* fruit is shown in Table 1. There was a marked ($p<0.05$) reduction in the percentage alternation in rats exposed to MSG alone compared with the saline-treated rats and other groups. The Co and pre-treatment with caffeic acid, luteolin and *P. dactyliferaa* then MSG caused significantly ($p<0.05$) increase in % alternation as compared to groups given MSG only.

Table 1:
The Mean and standard error of mean (SEM) of percentage alternation of Y maze

Experimental Groups	Percentage alternation
Control	90±2.2
MSG	45±1.3[a]
MSG+CAF	71±4.0[b]
MSG+LUT	68±3.3[b]
MSG+PD	72±4.7[b]
CAF--MSG	80±7.4[b]
LUT--MSG	85±4.7[b]
PD--MSG	82±3.0[b]

[a, b] signifies statistically significantly different as compared to control and MSG groups respectively

Cytoarchitecture of the Dentate Gyrus: The rats that were given normal saline, co treatment and pretreatment with luteolin, pretreatment with caffeic acid and *P. dactyliferaa* and MSG were dominated with intact granule cells (the nucleolus are centrally placed and cells are spherical in shape) while rats co-treated with caffeic acid and *P. dactyliferaa* with MSG were dominated with non-intact granule cells (the nucleolus cannot be visibly seen and the cell shape has lost is sphericity) and scanty intact granule cells. The MSG only group had predominantly non-intact granule cells as seen in Plate 1.

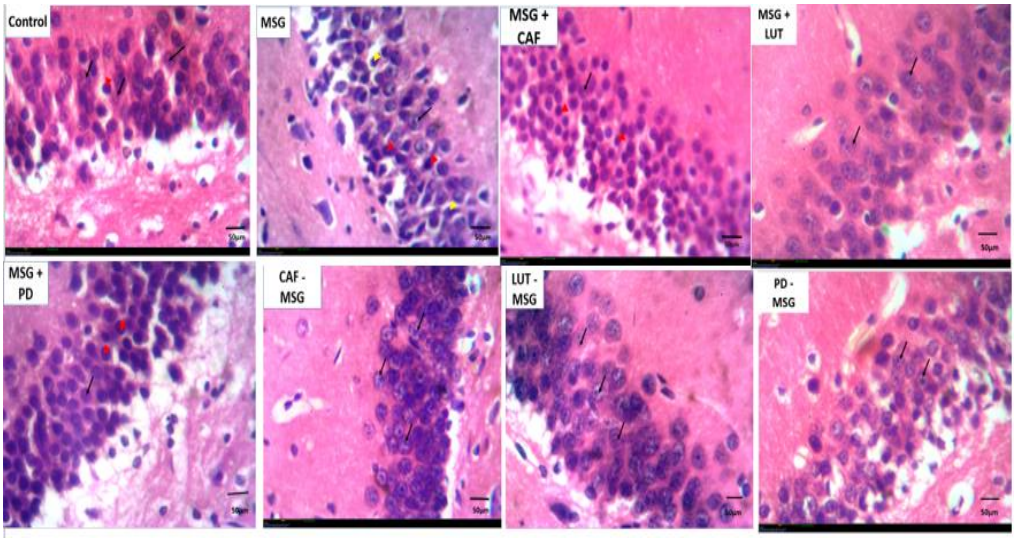

Plate 1:
Representative photomicrographs of the dentate gyrus of adult male Wistar rats showing the cytoarchitectural layer of its dentate gyrus with Hematoxylin and Eosin stain
Scale bars = 50µm. Magnification: x 400. Control, MSG = monosodium glutamate, LUT + MSG = Luteolin and MSG concurrently, MSG + PD = *Phoenix dactylifera* and MSG concurrently, MSG + CAF = caffeic acid and MSG concurrently, LUT – MSG = pre luteolin then MSG, PD – MSG = pre *Phoenix dactylifera* then MSG, CAF –MSG = pre caffeic acid then MSG. The black arrow indicates normal mature granule cells and red arrow head indicates immature granule cells.

Table 2:
The Mean and standard error of mean (SEM) of Hippocampal Malondialdehyde (MDA)Concentrations, Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) activities

Experimental groups	Malondialdehyde (U\ mg protein)	Superoxide Dismutase activities(U\mgprotein)	Glutathione Peroxidase(U\mg Protein)
Control	0.098±0.02	542±20	164±11
MSG	0.97±0.03[a]	50±9.6[a]	50±13[a]
MSG+CAF	0.81±0.07[a]	294±23[ab]	63±7[a]
MSG+LUT	0.33±0.05[ab]	164±4.7[ab]	101±8.7
MSG+PD	0.64±0.07[ab]	172±14[ab]	100±11
CAF--MSG	0.071±0.03[b]	458±24[b]	152±8.7[b]
LUT—MSG	0.16±0.01[b]	481±18[b]	138±5.1[b]
PD--MSG	0.32±0.03[ab]	450±9.6[b]	125±11

[a, b] signifies statistically significantly different as compared to control and MSG groups respectively.

Oxidative Stress Markers: The hippocampus of the experimental animals were isolated and homogenized in 5% sucrose. The concentration of malondialdehyde in the hippocampus of male Wistar rats after exposure to normal saline, monosodium glutamate, caffeic acid, luteolin, and *Phoenix dactylifera* fruit is shown (Table 2). There was a statistically significant ($p<0.05$) increment in the concentration of malondialdehyde in the hippocampus of rats exposed to MSG alone when compared with the saline-treated rats and other groups except the groups given concurrent treatment of caffeic acid and MSG. The administrations of caffeic acid, luteolin, and *P. dactyliferaa* before and/or concurrently exposed to MSG caused statistical significant decrease ($p<0.05$) in the concentration of malondialdehyde as compared to MSG only exposed rats. The activities of superoxide dismutase in the hippocampus of male Wistar rats after exposure to normal saline, monosodium glutamate, caffeic acid, luteolin and *Phoenix dactylifera* fruit is shown in Table 2. There was a significant ($p<0.05$) reduction in the activities of superoxide dismutase of rats exposed to MSG alone when compared with the saline-treated rats and other groups. The administrations of caffeic acid, luteolin, and *P. dactyliferaa* before and/or concurrently exposed to MSG caused significant increase ($p<0.05$) in the activities of superoxide dismutase as compared to the hippocampus of groups of rats exposed to MSG only.

The activities of glutathione peroxidase (GPx) in the hippocampus of male Wistar rats after exposure to normal saline, monosodium glutamate, caffeic acid, luteolin and *Phoenix dactylifera* fruit is shown in Table 2. There was a significant ($p<0.05$) reduction in the activities of GPx in rats exposed to MSG alone when compared with the saline treated rats, and other groups except the groups given concurrent treatment of caffeic acid and MSG. The administrations of caffeic acid, luteolin and *P. dactyliferaa* before and/or concurrently exposed to MSG caused significant increase ($p<0.05$) in the activities of glutathione peroxidase as compared to MSG only exposed rats.

DISCUSSION

Spatial memory was measured using percentage of spontaneous alternation. Deficit in spatial memory was indicated by significant reduction in percentage alternation in the MSG treated groups as compared to control. It has been reported that MSG reduces performance in spatial memory as well as the ability for learning task. This is as a result of over-excitation of the N-methyl-D- aspartate (NMDA) receptor (Swamy *et al*., 2013), thereby disrupting the normal course of long-term potentiation (Onaolapo *et al*. 2012).

However, significant increase in spatial memory was obtained in rats given caffeic acid, luteolin, and *P. dactyliferaa* before and/or concurrently exposed to MSG as compared to MSG only exposed rats. It has been reported that caffeic acid, luteolin, and *P. dactyliferaa* improves memory and learning due to their ability to enhance proliferation of granule cells in the hippocampal dentate gyrus. Their ability to exhibit neuroprotective and antioxidant effects are also contributory (Subash *et al*., 2015; Wang *et al*., 2016; Chang *et al*., 2019 and Zhou *et al*. 2019).

Neurogenesis occurs in the dentate gyrus even in adulthood (Hashem *et al*., 2010). It was observed that there was more immature granule cells in the MSG only as compared to control. It was reported that neuronal death within the hippocampus provide stimulus for increased dentate neurogenesis and it was established that dentate neurogenesis was reported in neurodegenerative diseases patients (Hashem *et al*., 2010). This may help compensate for the apoptotic cells during injury. However, pretreatment with caffeic acid, luteolin and *P. dactyliferaa* before MSG had dominant mature granule cells. The treatment exhibited protective effect on the granule cells.

The intensity of lipid peroxidation was accessed by measuring the level of MDA. Significant increase in level of lipid peroxidation in the rats given MSG was due to generation of reactive oxygen species (ROS). Malondialdehyde is a secondary resultant product of lipid peroxidation due to alteration in the membrane intergrity caused by tremendous increase in lipid peroxidation in the membrane which is dominated by polysaturated fatty acid (Fasakin *et al*., 2017; Arise *et al*., 2019). Its increase has been previously reported as a result of excitotoxicity (Arise *et al*., 2019). However, significant decrease in the extent of lipid peroxidation was apparent in rats given caffeic acid, luteolin, and *P. dactyliferaa* before and/or concurrently exposed to MSG as compared to MSG only exposed rats. These were ascertained by significant decrease in the level of MDA. It has been reported that caffeic acid and luteolin have stable chemical structure that aids its scavenging of ROS and is also effective in the scavenging of peroxyl radical involved in lipid peroxidation (Yucel *et al*., 2012; Agunloye and Oboh 2017 and Siddique *et al*., 2018)

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes are vital protective mechanisms against generated free radicals resulting from tissue damage. The SOD converts superoxide ion to less reactive hydrogen peroxide while GPx reduces lipid peroxide and hydrogen peroxides to lipid alcohols and water respectively. Reduction in the activities of the two enzymes could further help explain the increased lipid peroxidation in the MSG only group. Reduction in the activities of the two has been reported as a leading factor in oxidative stress, excitotoxicity and pathophysiology of neurodegenerative diseases (Onaolapo *et al*., 2016; Arise *et al*., 2019). However, significant decrease in the extent of oxidative stress was apparent in rats given caffeic acid, luteolin, and *P. dactyliferaa* before and/or concurrently exposed to MSG except the group given concurrent treatment of caffeic acid and MSG as compared to MSG only exposed rats. These were ascertained by significant increase in the level of SOD and GPX as compared to MSG only groups. It has been reported that caffeic acid, *Phoenix dactylifera* and luteolin help increase antioxidant capacity by upregulating the activities of SOD and GPx and scavenge or inhibit free radicals (Pujari *et al*., 2014; Alkis *et al*., 2015 and Akinrinde and Adebiyi, 2019). These results in the eventual protective effect being exhibited by caffeic acid, luteolin and Phoenix dactylifera.

In conclusion, this study demonstrated the potentials of luteolin, caffeic acid and *Phoenix dactylifera* on MSG-toxicity on the dentate gyrus. Luteolin, caffeic acid and *Phoenix dactylifera* had mitigating effects on the dentate gyrus cytoarchitecture, oxidative stress and working memory following the deleterious effect caused by MSG, with luteolin being the most effective treatment amongst the three. Pre-treatment with luteolin, caffeic acid and *Phoenix dactylifera* was more effective than cotreatment.

Conflict of Interest

There was no conflict of interest.

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Full length Research Article

Morphological, Biochemical and Molecular Docking Evaluation of the Anti-inflammatory Effects of Methanolic Extract of *Bridelia ferruginea* stem bark on Acetic acid-induced Ulcerative Colitis in Rats

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Summary: Ulcerative colitis (UC) is a chronic disorder that involves inflammation. This study was carried out to examine the anti-inflammatory effect of the methanol extract of *Bridelia ferruginea* stem bark in acetic acid-induced ulcerative colitis in male Wistar rats. Twenty-four rats were randomly divided into 6 groups of 4 animals each, colitis was thereafter induced by intrarectal administration of 4% (v/v) acetic acid in all except group 1, which received distilled water. For post-colitis induction treatment group 2 received distilled water, groups 3, 4 and 5 were orally administered the extract at doses of 100mg/kg, 200mg/kg and 400mg/kg, respectively while group 6 received sulfasalazine 500mg/kg orally. Post colitis induction, treatment lasted for 7 days and at the end of the experiment, colon samples were collected for estimation of antioxidant, inflammatory and histological parameters. Molecular docking study was also carried out to gain more insights about the promising anti-inflammatory compounds earlier identified in the extract. Results revealed that the extract significantly ($p<0.05$) attenuated the increased MDA, nitrite, TNF-α and IL-6 levels. Activities of SOD, CAT, MPO and GSH levels were also, significantly ($p<0.05$) increased. Furthermore, molecular docking study revealed that α-amyrin may have contributed significantly to the anti-inflammatory activity of the extract because of its remarkable binding affinity for IL-6, iNOS, IL1-β,TNF-α and COX-2 relative to prednisolone and celecoxib. This study suggests that the extract attenuated acetic acid-induced colitis via antioxidative and anti-inflammatory mechanisms.

Keywords*: acid, ulcerative colitis, Bridelia ferruginea, cytokines, molecular docking*

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INTRODUCTION

Ulcerative colitis (UC) is a chronic condition that causes inflammation and ulcers of the colon and rectum (Gisbert and Chaparro, 2019). It is an inflammatory bowel disease (IBD) with main symptoms being abdominal pain and diarrhea mixed with blood accompanied with weight loss, fever and anemia. Symptoms often appear gradually from mild to severe intermittent periods of no symptoms between flares. Complications may include abnormal dilation of the colon (megacolon), inflammation of the eye, joints, liver and colitis associated colon cancer (Wanderås *et al*., 2016; Greuter and Vavricka, 2019).

The manifestation of IBD globally was 59.25 per 100 000 people in 2019 (Wang *et al*., 2023). These figures are usually higher in western countries than other places in Africa (Alegbeleye, 2019; Hodges and Kelly, 2020). However, adoption of a western lifestyle (Zinocker and Lindseth, 2018; Hodges and Kelly, 2020) by Africans including Nigerians has increased the number of reported cases (Hodges and Kelly, 2020).

The etiology of the disease is unknown but some factors including genetics, environmental, microbial and immunological have been implicated in its pathogenesis, such that excessive immune responses initiate release of neutrophil infiltration, cytokines and other mediators which can cause damage to the colon tissues (Tian *et al.*, 2017; Gupta *et al*., 2022).

Medications such as the 5-amino salicylic acid (5-ASA) derivatives, steroids, antibodies, anti-tumor necrosis factor (TNF)-alpha and other agents that can crush immune responses have been helpful in the treatment of IBD (Lamb *et al*., 2019; Hermandez-Rocha and Vande, 2020) but they do not come without side effects in addition to the fact that some individuals do not respond to treatment with them (Papamichael and Cheifetz, 2019). Currently, there are no other therapeutic agents available to effectively suppress immune response, inflammation and oxidative stress in all individual patient (Cai *et al.*, 2021) and as such, it is

important to identify a better, yet effective agent with fewer and milder side effects in the management of ulcerative colitis.

Bridelia ferruginea (BF) is a plant of the family-*Euphorbiaceae*, genus–*Bridelia*, commonly found in Savannah regions. The Folkloric use of decoction from its various part in Africa includes purgative and vermifuge. Several studies have reported the anti-inflammatory properties and activities of *Bridelia ferruginea* stem bark (Akuodor *et al*., 2012; Oloyede *et al*., 2014). Akuodor *et al.*, (2011) reported the effects aqueous extract of BF on pain. Adetutu *et al*., (2011) also reported the antibacterial, antioxidant and healing properties of BF. In spite of the several ethnopharmacological reports on *Bridelia ferruginea*, its effects on colon inflammation are not known, hence the effect of its stem bark methanolic extract on acetic acid-induced colitis was investigated in male *Wistar* rats.

MATERIALS AND METHODS

Drugs and chemicals: acid (Qualikems, India), ketamine hydrochloride (Ciron Drugs & Pharmaceuticals, India), triochloro acid (TCA, Sigma, Germany), thiobarbituric acid (TBA, Sigma, Germany), 5, 50 dithio-bis-2-nitrobenzoicacid (DTNB, Sigma, Germany), hexadecyltrimethyl ammonium bromide (HTAB, Sigma, Germany) and Adrenaline (Sigma, Germany). Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were carried out by ELISA kits from Biolegend, USA.

Plant extraction: The plant extract was harvested at Oke-ata Abeokuta North, Abeokuta, Ogun state. Fresh bark peels of the plant were air-dried, pulverized and extracted exhaustively in methanol. The filtrate was concentrated and evaporated to dryness at 60°C with rotary evaporator (Stuart Barloworld, Model RE 300) and stored in a refrigerator until use for the experiments.

Animals: Male Wistar rats weighing 150 – 200 g were purchased from Mctemmy concepts laboratory animal for research. They were kept in cages and acclimatized for twenty-one days under normal environmental condition before the study commenced. The rats were allowed food and water freely. The norm of Laboratory Animal Care was strictly complied with throughout the experiment (Garber *et al.*, 2011).

Experimental design: Rats were divided into 6 groups as follows; Control group received no treatment throughout this study. Vehicle group, groups 3, 4, 5 and 6 received 1 ml of 4% (v/v) acetic acid through the rectum once for the generation of colitis (Fabia *et al*., 1992) (n = 5 animals per group). Groups 3, 4 and 5 were subsequently administered BfME at 100, 200, and 400 mg kg^{-1} respectively, per oral (p.o) for 7 days while group 6 was administered with sulfasalazine at 500 mg kg^{-1} (p.o) for 7 days. At the termination of the study, rats were euthanized with ketamine anesthetics (10 mg per kg intraperitonially). Colons from all rats were excised for antioxidant assays and histologic assessment.

Biochemical assessments of inflammation: Excised colon sections of rats were homogenized in sodium phosphate buffer (pH 7.4, 0.1M) and centrifuged at 4ºC at a speed of 1 x 10^4 rpm for 6 x10^2 secs to obtain the supernatants which were thereafter stored at -20ºC.

Estimation of malondialdehyde (MDA) concentrations in colon tissue homogenates: The MDA, an index of lipid peroxidation was estimated by the method of assay of thiobarbituric reacting substances (TBARs) (Nagababu *et al.,* 2010). In brief, 0.1mL of supernatant was diluted 20 times in 1.5x10^{-1}M Tris-KCl buffer, and deproteinized with 0.5mL trichloro acid (30%). The mixture was subsequently centrifuged at 4 x10^4 rpm for 6x10^3secs at room temperature. 0.2 mL of the supernatant was separated into Eppendorf tube, the 0.2 mL thiobarbituric acid (0.75%) was subsequently added and the combination was heated at 80ºC for 60 mins. The tubes were chilled with ice pack, then 0.2 mL of the cooled mixture was removed into a microplate reader (Labtech LT-4500, UK) and absorbance was measured at 532 nm. The result was calculated using an index of absorption for MDA (molar extinction coefficient 1.56 x 10^5/M/cm) and the concentration of TBARS in tissues were expressed as μmol MDA/mg protein.

Estimation of nitrite concentrations in colon tissue homogenates: Nitrite, as an indicator of nitric oxide (NO) production was measured according the method described by *(*Green *et al.*, 1982). Briefly, 0.1mL of the supernatant was added to a microtiter plate and incubated with 0.1mL freshly prepared Griess reagent for ten minutes at room temperature in the dark. Sodium nitrite (0-100 μM) was formulated as standard to obtain the standard curve. The absorbance was estimated at 540 nm in a microplate reader (Labtech LT-4500, UK). The concentration of nitrite was determined from sodium nitrite standard curve and expressed as μM nitrite/mg protein.

Estimation of glutathione (GSH) concentrations in colon tissue homogenates: Reduced glutathione (GSH) was estimated in the tissue supernatant (Jollow *et al.*, 1974). Briefly, 0.1mL of supernatant was diluted 20 times in 1.5 x 10^{-2} M Tris-KCl buffer, and deproteinzed with 0.5 mL trichloro acid (30%). The combination was centrifuged at 4 x10^4 rpm for 6 x 10^2 secs at room temperature. The 0.1 mL of the deproteinized supernatant was mixed with 0.1mL of 5^1, 5^1 -Dithios-nitrobenzoic acid (DTNB, 0.0006 M) in a microplate plate. The absorbance was read within five min at 405 nm in a microplate reader (Labtech LT-4500, UK). The glutathione concentration was deduced from standard curve of glutathione (0-200 μM) and expressed as a μM GSH/mg protein.

Assay of myeloperoxidase (MPO) activity in colon tissue homogenates: Myeloperoxidase was extracted from homogenized tissue by suspending the material in 0.5% hexadecyl trimethyl ammonium bromide (HTAB) in 50 mM potassium phosphate buffer, pH 6.0, before sonication in an ice bath for 10 seconds. The specimens were freeze-thawed 3 times, after which sonication was repeated. Suspensions were then centrifuged at 4 x 10^{-4} rpm for 15 min and the resulting supernatant was assayed spectrophotometrically for MPO. 0.1 ml of the supernatant was mixed with 290 uL of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% H_20_2.

The change in absorbance at 460 nm was estimated in a microplate reader (Labtech LT-4500, UK). One unit of MPO activity was defined as that degrading one micromole of H_20_2 per minute at 25°C (U/mg protein).

Assay of catalase activity in colon tissue homogenates: It was determined using the colorimetric assay founded on a complex formed with molybdate and H_2O_2 (Goth, 1991). In brief, 0.1mL diluted supernatant of the colon homogenates was introduced into a microplate reader, and thereafter 0.05 mL of the combination of H_2O_2 (65 mmol/mL) and Na^+-K^+ phosphate buffer (60 mM, pH 7.4) was added. The consequent reaction was incubated for 3 min and discontinued with 0.1 mL of ammonium molybdate (64.8 mM) in sulfuric acid. The absorbance at 405 nm was estimated in a microplate reader (Labtech LT-4500, UK). The enzyme activity unit was presented as U/mg protein.

Assay of superoxide dismutase (SOD) activity in colon tissue homogenates: The level of SOD activity was decided by the approach of Misra and Fridovich (Misra & Fridovich, 1972). Superoxide dismutase activity is established on its capability to prevent the autoxidation of adrenaline in sodium carbonate buffer (pH 10.7). 50 µL of supernatant diluted twice was introduced into a microtitre plate holding 0.15mL of carbonate buffer. Thereafter, 0.03mL of freshly prepared 0.3mM adrenaline was added to the mixture and the reaction. Blank was prepared using 0.05mL of distilled water. The rise in absorbance at 495 nm was observed every 60 seconds in four minutes. Activity was presented as U/mg protein.

Determination of tumor necrosis factor alpha (TNF-α) and Interleukin 6 (IL-6) levels in colon tissue homogenate: This was determined with the use of Biolegend ELISA kit, (USA) exclusive to the TNF-α and IL-6 with sensitivity constraint of 4 ρg/mL with the use of microplate reader of 450nm filter. Biolegend instructions were followed through in all the measurements at room temperature. The concentration of the cytokines was extrapolated from the curves of IL-6 and TNF-α standards contained in the assay kits and subsequently expressed as ρg/mL.

Histologic assessment of the colon: Samples of the colon tissue were processed for colitis estimation by an investigator shaded to study design and analysis was described (Owen *et al*., 2011). In brief, the tissues were fixed in formalin (10%). Water was removed in graded alcohol. Thereafter, it was cleared in xylene and fixed in paraffin wax. The tissues were later cut into sections (four micrometer thick) by a microtome, embedded on the slides and stained with hematoxylin and eosin (H&E). The resultant slides were examined underneath a light microscope (Olympus, Japan) and photomicrographs were taken with DM750 camera (Leica, Germany) at 100 magnifications.

Statistical Analysis: Results were presented as mean ± SEM. Data were compared for significant main effect using analysis of variance(one-way) and followed by Newman–Keuls post-hoc multiple-comparison test (unless otherwise stated). Data analysis and graph plots were performed with GraphPad 5.0 software (GraphPad Software Inc). Values were considered statistically significant when $p < 0.05$.

Protein preparation: The crystal structures of IL-6 (PDB ID of 1ALU), TNF-α (PDB ID of 2AZ5), iNOS (PDB ID of 1r35) IL1B (PDB ID of 9ILB) COX 2 (PDB ID of 3LN1) were recalled from the protein data records (http://www.rcsb.org). The protein structures were prepared by removing bound ligands and water molecules while the required hydrogen atoms were added by using Discovery Studio, 2021. Using PyRx 0.8, the proteins were converted to Protein Data Bank, Partial Charge, and Atom Type (PDBQT) format to be used for molecular docking.

Ligand preparation: The structure data file (SDF) format of prednisolone, celecoxib, and the seventeen most abundant compounds identified in *B. ferruginea* methanolic extract in earlier study (Omolaso *et al*., 2021), were retrieved from http://www.pubchem.ncbi.nlm.nih.gov, (PubChem database). Open Babel built into PyRx 0.8 was operated to convert the ligands into PDBQT format to be used for molecular docking, using Vina (Trott & Olson, 2010)

Molecular Docking: Vina (Trott & Olson, 2010) incorporated into PyRx 0.8 was employed for docking and binding affinity determination. The individual enzyme in PDB format were brought in into the PyRx and changed to PDBQT, while the ligands were imported through Open babel(O'Boyle et al., 2011)where energy minimization before conversion to PDBQT format. A cluster analysis based on RMSD values, with reference to the starting geometry was subsequently executed and the minimum energy conformation of the most populated cluster was taken as the most reliable solution. The binding energy of the ligands for the five targets were documented and then ranked by their affinity values. Molecular interactions between the outstanding compound and the protein targets were viewed with the aid of Discovery Studio Visualizer, BIOVIA, 2021.

RESULTS

***Bridelia ferruginea* methanol extract (BfME) improves macroscopic signs of experimental colitis:** Control rat's colon appeared normal macroscopically, intra-rectal administration of acetic acid in the vehicle treated rats however, caused colonic inflammation evidenced by hyperemia, edema and ulcers. On the contrary, rats administered with BfME at 100, 200, and 400 mg kg^{-1} showed mild edema, colonic inflammation, thickening and ulceration while sulfasalazine treated rats showed similar macroscopic appearance as seen in rats given BfME with moderate colonic inflammation, edema, ulceration and less thickening (Figure 1).

***Bridelia ferruginea* methanol extract (BfME) reduced Malondialdehyde (MDA) levels during experimental colitis in rats:** The mean values of MDA levels in rat's colon tissue homogenate post intrarectal administration of acetic acid are presented on Figure 1.

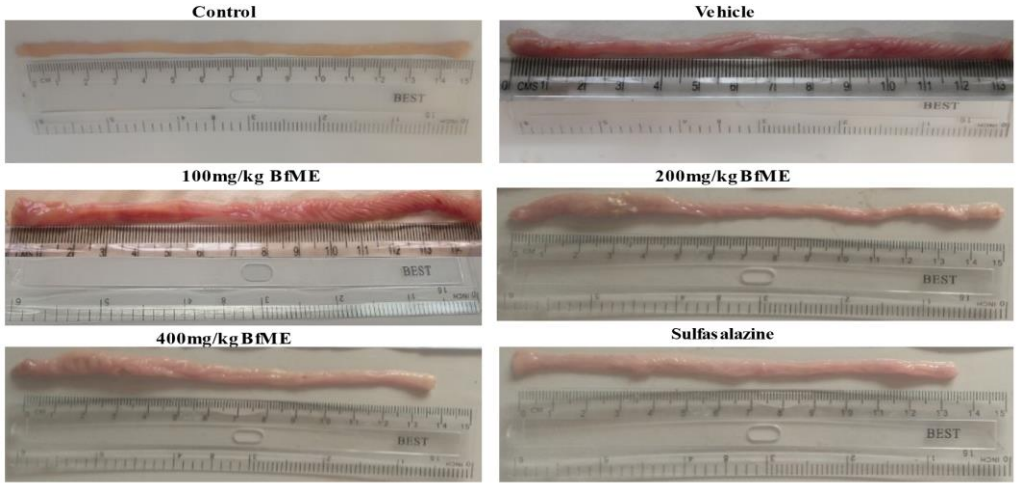

Plate 1:
Macroscopic appearance of colon tissues 8 days post intra-rectal challenge with 4% (v/v) acid and oral administration BfME (100, 200 and 400 mg kg^{-1}) and sulfasalazine (500 mg kg^{-1}).

The generation of colitis with acetic acid was followed by significant rise in mean MDA level to 15.91 ± 0.29 nmol/mg protein in vehicle group compared to the control group which was 6.75±0.74 nmol/mg protein. The MDA level decreased significantly ($p < 0.05$) in rats treated with BfME at 100, 200, and 400 mg kg^{-1}; mean values are12.42 ± 0.62, 10.70 ± 0.71, and 10.06 ± 0.67nmol/mg protein respectively, when compared with rats in the vehicle group. Sulfasalazine also, decreased mean MDA level to 10.52 ± 0.33 nmol/mg protein compared to Vehicle.

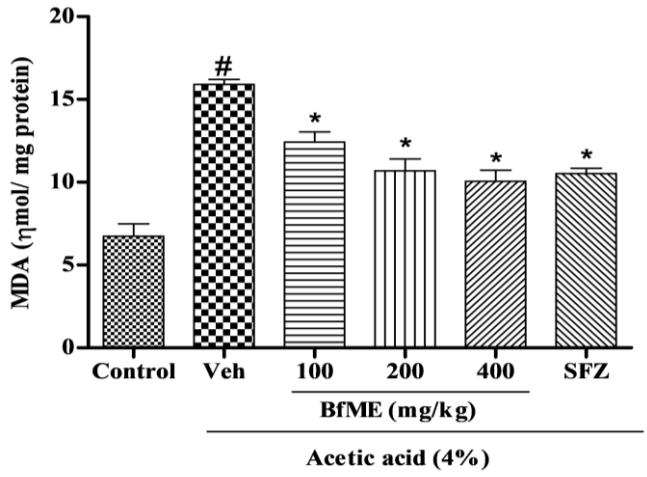

Figure 1
Quantification of MDA levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

***Bridelia ferruginea* methanol extract (BfME) reduced nitrite levels during experimental colitis:** The mean values of nitrite level in rat's colon tissue homogenate post intrarectal administration of acetic acid are presented on Fig. 2. Colitis induction with acetic acid was attended with significant rise in nitrite levels. The value of Nitrite level in tissue homogenate was significantly ($p < 0.05$) raised in the Vehicle treated rats (mean value 5.34 ± 0.44 nmol/mg protein) when compared with values obtained in the control group (mean value 2.38± 0.14 nmol/mg). BfME treatment at 100, 200, and 400 mg kg^{-1} prevented the increase in the Nitrite level with mean values being 3.26 ± 0.33, 2.68 ± 0.24, and 2.58 ± 0.15 nmol mg^{-1} protein, respectively, matched with values in vehicle treated animals. Sulfasalazine significantly reduced mean of the level of nitrite to 2.48 ± 0.17 nmolmg^{-1} protein when compared to Vehicle.

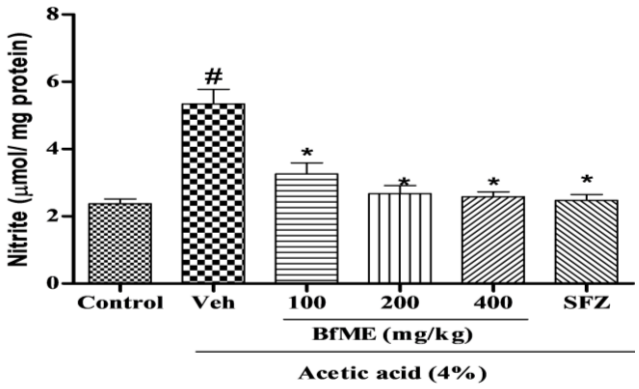

Figure 2.:
Quantification of nitric oxide (NO) levels during experimental colitis in rats.
Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

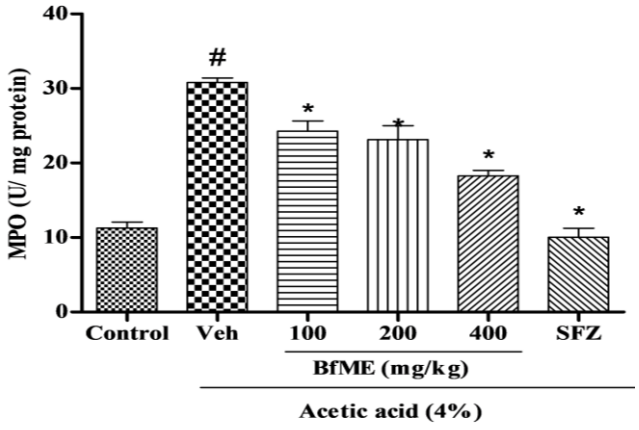

Figure. 3:
Quantification of myeloperoxidase (MPO) activity during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

***Bridelia ferruginea* methanol extract (BfME) reduced myeloperoxidase (MPO) activity during experimental colitis:** The mean values of MPO activity in rat's colon tissue homogenate post intrarectal administration of acetic acid are presented on Fig. 3. Mean MPO activity was raised significantly to 30.83 ± 0.58 Umg^{-1} protein in the Vehicle group when matched with values in the Control group which had mean MPO activity values as 11.27± 0.83U/mg protein. BfME at 100, 200, and 400 mg kg^{-1}significantly attenuated MPO activity in that it reduced MPO activity values to 24.29 ± 1.37, 23.16 ± 1.84, and 18.28 ± 0.75 U/mg protein respectively, compared to vehicle. Sulfasalazine significantly decreased mean MPO activity to 10.04 ± 1.21 U/mg protein compared to Vehicle.

***Bridelia ferruginea* methanol extract (BfME) improved reduced glutathione (GSH) concentration during experimental colitis:** The mean values of GSH concentration in rat's colon tissue homogenate after administration of acid via the rectum are presented on Fig. 4. Intrarectal administration of acid in the Vehicle resulted in significantly decreased GSH concentration; 1.95 ± 0.15 nmol/mg protein compared with Control which was 4.15± 0.42nmol/mg protein. Mean GSH concentration post intrarectal administration of acid in rats administered with BfME at a dosage of 100 mg $kg^{-1}$was 2.39 ± 0.23, and in rats administered with BfME at dosages of 200 and 400 mg kg^{-1}, GSH concentration significantly increased to 5.98 ± 0.55 and 6.64 ± 0.78 nmol/mg protein respectively, compared to Vehicle. Sulfasalazine significantly increased mean GSH concentration to 10.13 ± 0.20 nmol/mg protein compared to Vehicle.

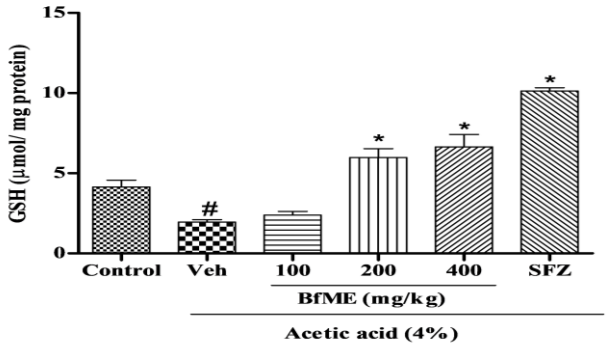

Figure 4:
Quantification of glutathione (GSH) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

***Bridelia ferruginea* methanol extract (BfME) improved Superoxide dismutase (SOD) activity during experimental colitis**: The mean values of SOD activity in rat's colon tissue homogenate following intrarectal administration of acetic acetic acid is presented on Figs. 5. Colitis induction was followed with significant reduction of superoxide dismutase (SOD) enzyme activity in control group; mean value being 0.39± 0.03Umg^{-1} protein. acid administered via the rectum significantly reduced the mean activity level of SOD to 0.21 ± 0.01 Umg^{-1} protein when matched with values observed in the control group, BfME significantly increased mean SOD activity to 0.34 ± 0.01, 0.34 ± 0.02, and 0.31 ± 0.01 U/mg protein at the dosages of 100, 200, and 400 mg/kg, compared to vehicle. Sulfasalazine also, significantly raised mean value of SOD activity to 0.40 ± 0.03 U/mg protein compared to Vehicle.

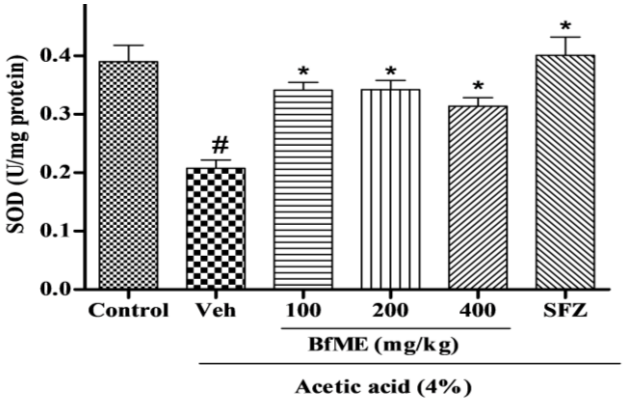

Figure 5:
Quantification of superoxide dismutase (SOD) activity during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

***Bridelia ferruginea* methanol extract (BfME) improved Catalase (CAT) activity during experimental colitis**
The mean values of CAT activity in rat's colon tissue homogenate post administration of acetic acid is presented on Figs. 6. Mean activity of catalase (CAT) in colon tissue homogenate of control group was 7.09 ± 0.13Umg-1 protein. acid significantly decreased the mean CAT activity in vehicle treated rats to 3.85 ± 0.36 Umg^{-1} protein when matched with values obtained in the control animals. BfME significantly raised mean activity of CAT to 5.58 ± 0.34, 5.60 ± 0.49, and 6.06 ± 0.40 Umg^{-1} protein at dosages of 100, 200, and 400 mg kg^{-1} respectively, matched to vehicle. Sulfasalazine also, significantly increased mean CAT activity to 5.99 ± 0.42 U/mg protein compared to Vehicle.

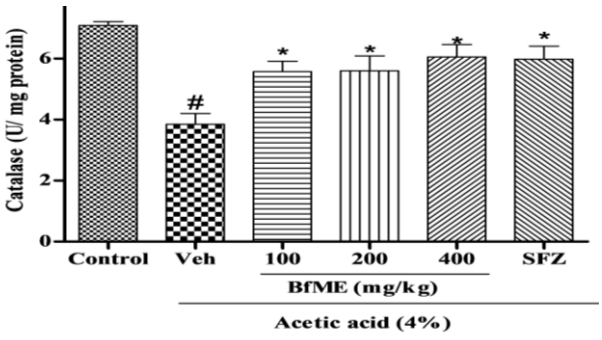

Figure 6:
Quantification of catalase (CAT) activity during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

***Bridelia ferruginea* methanol extract (BfME) reduced TNF-*α* level during experimental colitis.**
The mean values of TNF-*α* level in rat's colon tissue homogenate post intrarectal administration of acetic acid are presented on Figs. 7. The generation of colitis with acetic acid was followed by significant rise in TNF-*α* such that mean TNF-*α* level in the vehicle group was 3.52 ± 0.27 pg/mg protein compared to the control group which was

1.13 ± 0.86 pg/mg protein. BfME only slightly decreased mean TNF-α level to 2.84 ± 0.32 pg/mg protein at 100 mg kg^{-1} dose, but at 200 and 400 mg kg^{-1} dosages, TNF-α levels significantly reduced to 1.82 ± 0.13 and 1.83 ± 0.08 pg/mg protein respectively, compared to Vehicle. Sulfasalazine also significantly reduced mean level of TNF-α to 1.69 ± 0.20pgmg^{-1} protein when matched with Vehicle.

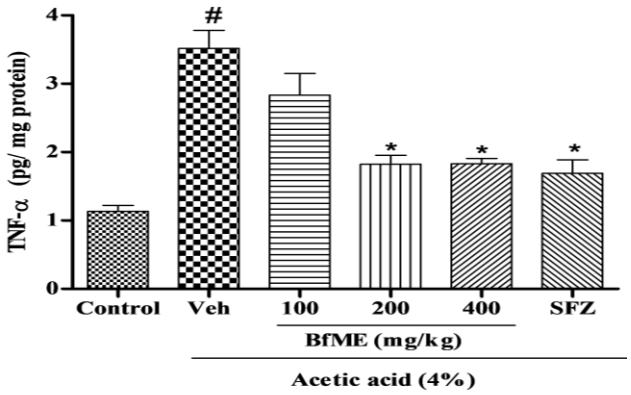

Figure 7:
Quantification of tumor necrosis factor alpha (TNF-α) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

***Bridelia ferruginea* methanol extract (BfME) reduced I L-6 level during experimental colitis**

The mean values of IL-6levels in rat's colon tissue homogenate post intrarectal administration of acetic acid acid are presented on Fig. 8. The mean value of IL-6 in the colon tissue homogenate of control group was 4.29 ± 0.31 pg/mg protein. acid significantly increased mean IL-6 level to 8.72 ± 0.48 pg/mg protein in the Vehicle treated rats when compared with values in the control animals. BfME significantly decreased mean IL-6 levels to 6.80 ± 0.24,6.77 ± 0.36, 6.42 ± 0.33 pgmg^{-1} protein at 100, 200 and 400 mg/kg dosages respectively, matched to vehicle. Sulfasalazine decreased mean IL-6 level to 6.10 ± 0.63 pg/mg protein compared to vehicle.

***Bridelia ferruginea* methanol extract (BfME) improved histopathological observations during experimental colitis**: Presented in Fig 9 are the representative micrographs of colon sections in the control, vehicle, 100, 200 and 400 mg kg^{-1} dosage groups respectively. Histology of the colon sections of control rats had an intact normal structure with, muscularis propria submucosa, laminal propria, and epithelial architecture unbroken. In divergence, intrarectal administration of acetic acid caused stern epithelial loss, necrotic and warped cryptic glands that was also, associated with marked mononuclear cells intrusion and deteriorating changes in the colon sections. Administration of BfME and the standard medication, Sulfasalazine however, renewed the functional cellular structure of the mucosa of the colon reducing inflammatory cellular penetration, hyperplasia, ulceration and necrosis affected by acetic acid as evidenced by the histopathological assessment.

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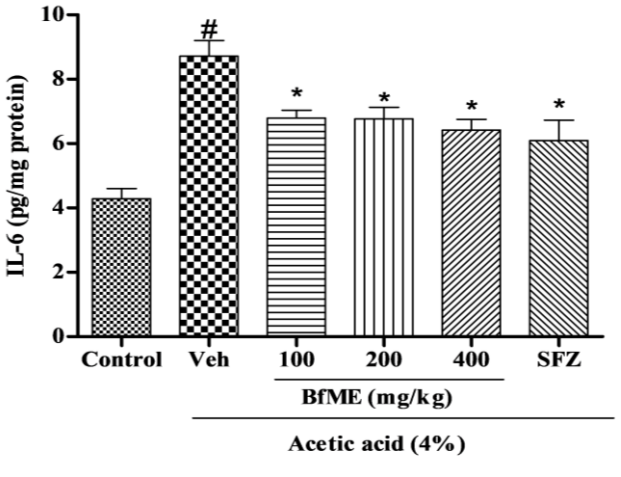

Figure 8:
Quantification of interleukin 6 (IL-6) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control ($p < 0.05$); * = significantly different from Vehicle ($p < 0.05$), as determined by one-way ANOVA.

Molecular docking

For IL-6, the reference anti-inflammatory standard drugs prednisolone (binding energy of -5.6 kcal/mol) and celecoxib (binding energy of -5.7 kcal/mol) individually showed considerable binding energy, however, γ-sitosterol, α-amyrin, and stigmasterol, performed better than these standards with respective binding energy of -6.0 kcal/mol, -6.7 kcal/mol, and -5.8 kcal/mol respectively (Table 1). For iNOS, prednisolone displayed a binding energy of -6.5 kcal/mol, celecoxib displayed a binding energy of -7.8 kcal/mol, while α-amyrin showed a considerable binding energy of -8.1 kcal/mol (Table 1). For IL1-β, γ-sitosterol and α-amyrin with respective binding energy of -7.5 kcal/mol and -7.2 kcal/mol performed better than the two standards prednisolone and celecoxib which displayed -3.8 kcal/mol and -6.8 kcal/mol binding energy respectively (Table 1). With respect to TNF-α, γ-sitosterol, α-amyrin and stigmasterol also performed better than the standards with respective binding energy of -8.7 kcal/mol, -10.2 kcal/mol, and -8.3 kcal/mol respectively relative to -8.0 kcal/mol and -8.1 kcal/mol for prednisolone and celecoxib respectively (Table 1). In the case of COX-2, α-amyrin also displayed a higher binding energy of -7.7 kcal/mol relative to -7.4 kcal/mol and -6.9 kcal/mol for prednisolone and celecoxib respectively. These results showed that α-amyrin displayed higher binding affinity for all the five target proteins compared to prednisolone and celecoxib.

The interaction pattern of celecoxib with IL-6 include hydrogen bond with residue Lys-120, hydrogen bond with Glu-99, Pro-141, Asn-144Tyr-346, py-alkyl bond with Leu-92, Pro-139, Ala-145 and Leu-148, while α-amyrin interacted with Leu-147 via py-alky bond (Plate 3).

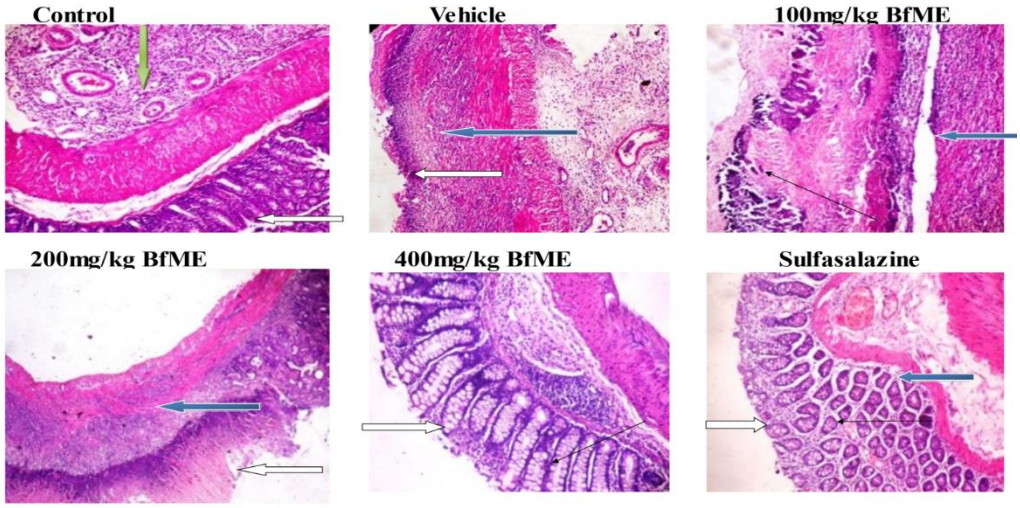

Plate 2
Representative H&E micrographs showing colon mucosa on day 9.
Control shows a preserved mucosal epithelium in contrast Vehicle that is showing poorly preserved mucosa epithelium; ulcer and necrosis (white arrow), lamina propria shows suppurating inflammation and necrosis, the sub mucosal layer and muscularis layer show infiltration of inflammatory cells (blue arrow). Administration of BfME at the dosages of 200 and 400 mg kg^{-1}resulted in an improved epithelium as evidenced by a mild to moderately preserved mucosal epithelial layer, lamina propria is however, infiltrated by inflammatory cells. Sulfasalazine treatment also, attenuated inflammation as revealed by the moderately preserved mucosal epithelial layer (white arrow), lamina propria is however, infiltrated by inflammatory cells (slender arrow), the sub mucosal layer shows mild infiltration of inflammatory cells (blue arrow) and the serosal layer is also, inflamed and well vascularized (H & E Stain; *100).

Table 1:
Docking results of major compounds identified in *Bridelia ferruginea* methanol extract with selected anti-inflammatory protein targets

S/N	Compounds	Binding affinity (kcal/mol)				
		IL-6	iNOS	IL1-β	TNF-α	COX-2
S	Prednisolone	-5.6	-6.5	-3.8	-8.0	-7.4
S	Celecoxib	-5.7	-7.8	-6.8	-8.1	-6.9
1	Octadecenoic acid	-4.7	-3.9	-4.7	-5.2	-4.4
2	n-Hexadecenoic acid	-4.5	-4.8	-4.3	-5.3	-4.1
3	Methyl-11-octadecenoate	-4.3	-4.4	-3.5	-5.1	-4.5
4	Cis-13-Eicosenoic acid	-4.8	-4.6	-4.3	-5.4	-4.1
5	cis-11-Eicosenoic acid, methyl ester	-3.9	-4.7	-3.9	-5.4	-4.4
6	Methylhexadecanoate	-3.6	-4.6	-4.6	-5.3	-4.2
7	Eicosanoic acid	-4.4	-4.5	-4.2	-5.6	-4.1
8	Octadecanoic acid	-4.1	-4.8	-4.4	-5.0	-4.3
9	γ-Sitosterol	**-6.0**	-7.6	**7.5**	**-8.7**	-7.1
10	Methyl 18-methylnonadecanoate	-4.6	-4.4	-3.7	-5.1	-4.4
11	13-Docosenoic acid, methyl ester, (Z)-	-4.1	-4.3	-3.6	-5.4	-4.2
12	α-Amyrin	**-6.7**	**-8.1**	**7.2**	**-10.2**	**-7.7**
13	Methyl stearate	-4.2	-4.5	-3.9	-4.8	-3.9
14	Stigmasterol	**-5.8**	-7.4	-4.0	**-8.3**	-6.6
15	2,3-Dihydroxypropyl elaidate	-4.9	-4.9	-4.0	-5.5	-4.5
16	Erucic acid	-4.0	-5.4	-3.8	-5.2	-4.1
17	Tetradecanoic acid	-4.0	-4.8	-4.6	-4.8	-4.2

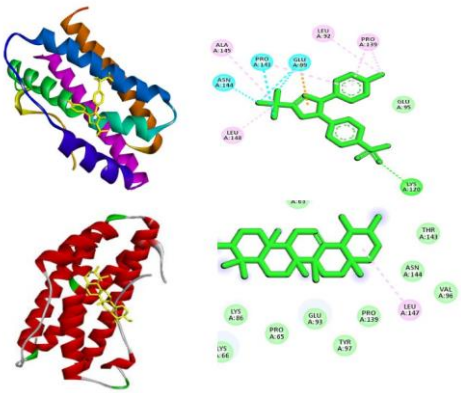

Plate 3:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) with IL-6

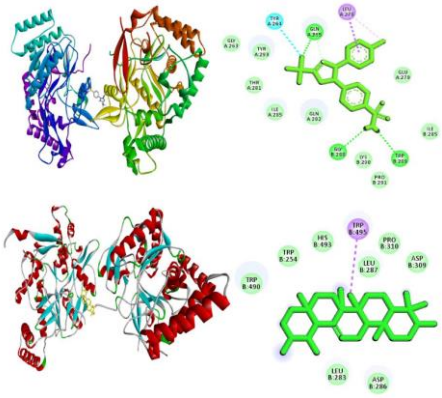

Plate 4:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) iNOS

The interaction pattern of celecoxib with IL-6 includes hydrogen bond with residue Gln-265, Gly-288 and Trp-289, halogen bond with Tyr-264, py-sigma bond with Leu-287 while α-amyrin interacted with Trp-495 via pi-sigma (Plate 3). Also, Plate 5 shows the interaction pattern of celecoxib with IL-β include hydrogen bond with residue Leu-26, Leu-80, Leu-134, halogen bond with Tyr-24, Leu-80 and Val-132, pi-sigma bond with Leu-80, Pro-131 while α-amyrin interacted with Met-20 via pi-alkyl.

The interaction pattern of celecoxib with TNF-α was dominated by hydrogen bond, pi-alkyl bonds and halogen bond while only van der Waals forces could be observed for α-amyrin (Plate 6).

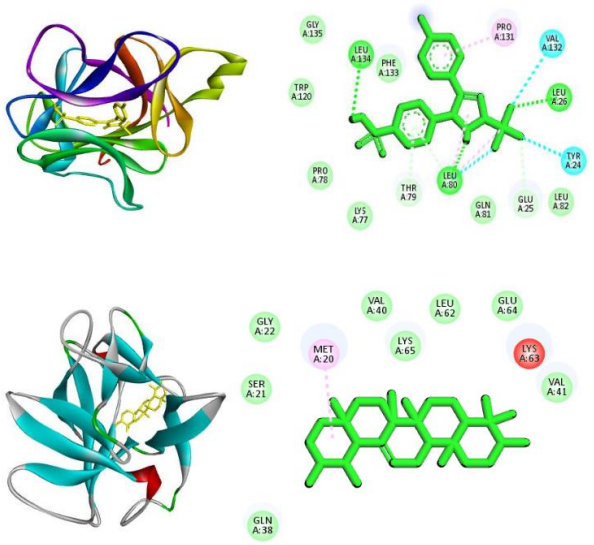

Plate 5:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) IL1-β (alkyl)

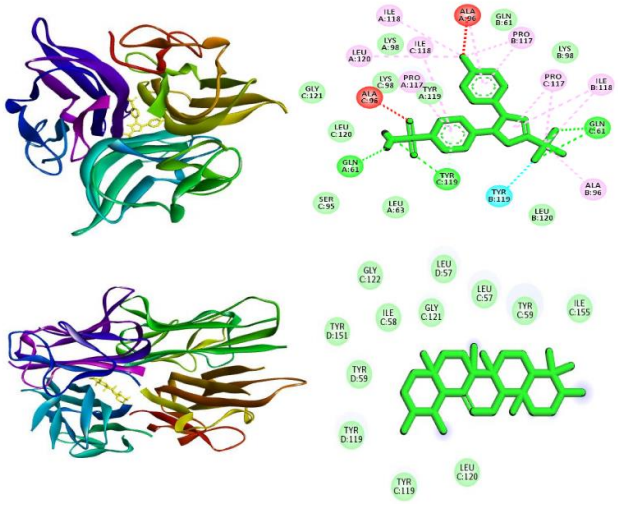

Plate 6:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) TNF-α

The interaction pattern of celecoxib with COX-2 include hydrogen bond with residue Lys-239, Thr-255, Gln-256, Arg-297 and Thr-547, halogen bond with Lys-543, pi-sigma bond with Leu-232 while α-amyrin interacted with Ile-301 via pi-alkyl (Plate 7)

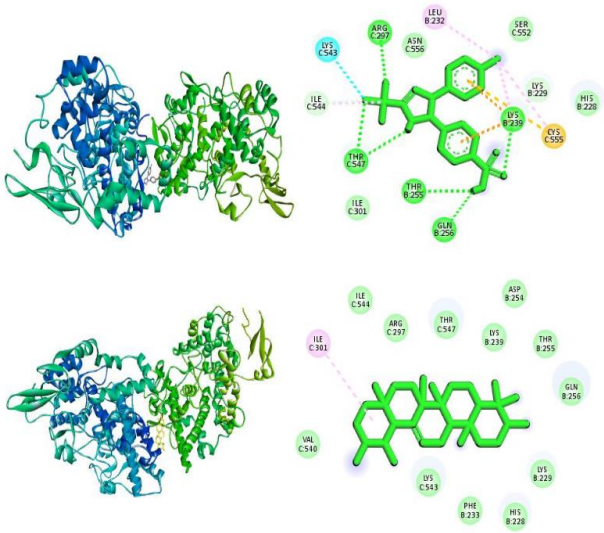

Plate 7:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) COX-2

DISCUSSION

Induction of experimental ulcerative colitis by low application of acid via the rectum in rodents is recognizable simulation for studying IBD (George *et al.*, 2020). Although this model and human IBD vary in etiology, they both have mutual pathophysiological descriptions and medication response in the course of treatment. Changes such as weight loss ulceration of the mucosa, blood loss and inflammation are examples of features that accompany them (Misbahuddin *et al.*, 2021). Also, there is influx of inflammatory cells, rupture of colon mucosal barrier, release of inflammatory mediators and the production of reactive oxygen species (ROS) in both acid-induced colitis and IBD which results in decreased antioxidant system activity and consequently oxidative stress (Rehman *et al.*, 2022; Sahoo *et al*., 2023).

In the present study, colitis was induced by intrarectal administration of 1 ml of acetic acid (4% *v/v*). Administration of vehicle (distilled water), BfME at 100, 200 and 400 mg kg^{-1}, and sulfasalazine (500 mg kg^{-1}) was done appropriately according to rat grouping and these commenced on the 3rd day post colitis induction till the 9th day when rats were euthanized.

In this study, significant inhibition of MDA levels in colon tissue homogenate of rats administered with BfME is indicative of its antioxidant and anti-inflammatory potentials. MDA, a by-product of lipid peroxidation occurring in tissues increases in inflammatory conditions such as in ulcerative colitis and thus, represent an important marker in the diagnosis of colitis (Rehman *et al.*, 2022). Since lipid peroxidation increases during oxidative stress, administration of plant products such as BfME may be beneficial in synergy with endogenous antioxidant enzyme activities in reducing the production of oxidants.

Induction of colitis elevated the concentration of NO in this study; this was evidenced in the vehicle group. Administration of BfME however, significantly subdued NO generation which may have averted peroxynitrite reduction from inflammatory cells with consequent

reduction in inflammation. Same effect of BfME on NO concentration in colon tissue homogenate of rats was observed with sulfasalazine administration. NO is considered a pro-inflammatory mediator when it is overproduced by means of inducible NO synthase. In such circumstances it can further increase inflammation to form a peroxynitrite (ONOO–), a powerful oxidant, through its reaction with superoxide anion (O^{2-}) (Cross & Wilson, 2003). The acetic acid mediated increase in NO concentration was apparently suppressed by BfME in this study and suggests the extract's ability to prevent neutrophil infiltration and inflammation in the colon as observed in the photomicrographs.

Myeloperoxidase (MPO) is a pro-inflammatory enzyme found in azurophilic granules of neutrophilic granulocytes which during stimulation produces highly reactive oxidants and cross-link proteins (Hoskin *et al*., 2019). BfME significantly attenuated the MPO activity, indicating the extract possesses anti-inflammatory properties that can target MPO during colitis, an action that may make it a potentially suitable therapeutic agent.

Reduced glutathione (GSH) is a vital antioxidant mediator useful in the removal of free radicals that can cause mucosal damage (Vázquez-Meza *et al*, 2023). It plays crucial role in preserving the integrity of the epithelium against inflammation (Yajie *et al*., 2020). During inflammation there is oxidative stress and this causes a reduction in GSH concentration (Kumar *et al*., 2022).In this study, the induction of colitis with acid resulted in reduction of colonic tissue GSH concentration in rats administered with vehicle. However, BfME administration significantly attenuated the reduction in colonic tissue GSH concentration. The improvement of GSH concentration in rats administered with BfME may be indicative of its potentials for mopping up reactive oxygen species which in turn could attenuate acetic acid-induced oxidative damage in colon tissues.

In normal conditions, SOD and CAT are crucial in defense against oxidative stress in various disease conditions. In UC, activities of these duo in the tissues of the colon turn out to be intense and consequently they become depleted due to damage caused by free radicals (Vona *et al*., 2021). SOD protects colon tissues against colitis and consequently disallowed lipid peroxidation through the prevention of transformation of superoxide anion (Alyaa *et al.*, 2021). SOD also disallowed leukocyte sticking together and penetration in the colon and as for CAT, it is concentrated in peroxisomes and they catalyze the change of H_2O_2, a cytotoxic agent to H_2O and O_2 (Baldo& Serrano, 2017). Administration of BfME prevented exhaustion of SOD and CAT activities thus, leading to overall protection of colonic tissues post acetic acid-induced experimental colitis. The opposite was however, observed in the rats that were administered with the vehicle.

Tumor necrosis factor alpha and IL-6 are inflammatory cytokines that play very important role in the pathophysiology of inflammatory conditions as they regulate mucosal immunity by altering the functions of the epithelium, activation and enhancement of cellular infiltrates like neutrophils and macrophages which then result in mucosal damage of the colon (Jang *et al*, 2021). The elevated values of IL-6 and TNF-*α* in rats administered with vehicle in contrast to their low values in rats administered with BfME further supports its anti-inflammatory potentials. A similar observation of low IL-6 and TNF-*α* values in rats administered with BfME was also noticed in the sulfasalazine group.

Histopathological assessment revealed that administration of BfME preserved the structural architecture and function of the intestinal colonic mucosa epithelium by disallowing inflammatory cell penetration, necrosis, hyperplasia ulceration triggered off by acetic acid, an indication of the ability of BfME to prevent progression of colonic inflammation.

Studies on molecular docking were carried out at the later end of the experiments to gain more insights about the promising activity profile of the compounds earlier identified in BfME (Omolaso *et al.,* 2021). Results from the docking study revealed outstanding binding affinity of α-amyrin for all evaluated anti-inflammatory protein targets, exhibiting superior binding affinity for IL-6, iNOS, IL1-β, TNF-α and COX-2 relative to prednisolone and celecoxib. Interestingly, earlier studies have established the anti-inflammatory activity of this pentacyclic triterpene. For example, α-amyrin and β-amyrin acetate isolated from the stem bark of *Alstonia boonei* displayed significant anti-inflammatory activity in egg albumen-induced paw edema and xylene- induced ear edema models in experimental animals (Akindele *et al*., 2020). Vitor and colleagues have also demonstrated the anti-inflammatory effect of α-amyrin in trinitrobenzene sulphonic acid-induced colitis in mice, relative to dexamethasone (Vitor *et al*., 2009), while Medeiros *et al*., highlighted its anti-inflammatory effect on phorbol ester 12-O-tetradecanoylphorbol-13-acetate-induced skin inflammation of mouse skin (Medeiros et al., 2007). Stigmasterol displayed notable binding affinity with IL-6 and TNF-α while γ-Sitosterol showed notable binding affinity with IL-6, IL1-β and TNF-α. These compounds could be the major active components responsible for the observed anti-inflammatory activity of BfME in this study.

In conclusion, *Bridelia ferruginea* stem bark methanolic extract demonstrated antioxidative and anti-inflammatory activities in acetic acid-induced rats by restoring tissue levels of NO, MDA, GSH, TNF-α, IL-6, and activities of MPO, SOD and CAT. Furthermore, α-amyrin from the methanolic extract showed various levels of binding affinities and molecular interactions with anti-inflammatory protein targets, including IL-6, iNOS, IL1-β, TNF-α and COX-2. These results may justify the folkloric use of this plant in the treatment of inflammatory conditions in traditional medicine.

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Full length Research Article

Cajanus cajan (L) Millsp. Seed Extract Ameliorates Scopolamine-Induced Amnesia through Increase in Antioxidant Defense Mechanisms and Cholinergic Neurotransmission

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Summary: Background: Decline in cholinergic function and oxidative/nitrosative stress play a central role in Alzheimer's disease (AD). Previous quantitative HPLC profiling analysis has revealed the presence of Pinostrobin, formononetin, vitexin and other neuroprotective flavonoids in *Cajanus cajan* seed extract. Objective: This study was designed to investigate the protective action of *Cajanus cajan* ethanol seed extract (CC) on learning and memory functions using scopolamine mouse model of amnesia. Materials and methods: Adult mice were pretreated with CC (50, 100, or 200mg/kg, p.o) or vehicle (10ml/kg, p.o) for 16 days consecutively. Scopolamine, a competitive muscarinic cholinergic receptor antagonist (1mg/kg, i.p.) was given an hour after CC pretreatment from days 3 to 16. The mice were subjected to behavioural tests from day 11 (open field test (OFT)/ Y-maze test (YMT) and Morris water maze task (MWM) from days 12-16. Animals were euthanized 1h after behavioral test on day 16 and discrete brain regions isolated for markers of oxidative stress and cholinergic signaling. Molecular docking analysis was undertaken to predict the possible mechanism(s) of CC-induced anti-amnesic action. Results: pre-administration of CC significantly reversed working memory and learning deficits caused by scopolamine in YMT and MWM tests, respectively. Moreover, CC prevented scopolamine-induced oxidative and nitrosative stress radicals in the hippocampus evidenced in significant increase in glutathione (GSH) level, superoxide dismutase (SOD) and catalase (CAT) activities with a marked decrease in malondialdehyde (MDA) production as well as significant inhibition of hippocampal scopolamine-induced increase in acetylcholinesterase activity by CC. The molecular docking analysis showed that out of the 19 compounds, the following had the highest binding affinity; Pinostrobin (-8.7 Kcal/mol), friedeline (-7.5kCal/mol), and lupeol (-8.2 Kcal/mol), respectively, to neuronal muscarinic M1 acetylcholine receptor, α7 nicotinic acetylcholine receptor and amyloid beta peptide binding pockets, which further supports the ability of CC to enhance neuronal cholinergic signaling and possible inhibition of amyloid beta aggregation. Conclusion: this study showed that *Cajanus cajan* seeds extract improved working memory and learning through enhancement of cholinergic signaling, antioxidant capacity and reduction in amyloidogenesis.

Keywords*: amyloid beta peptide; α7 nicotinic acetylcholine receptor; Cajanus cajan; molecular docking; M1 muscarinic acetylcholine receptor; oxidative stress*

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INTRODUCTION

Alzheimer's disease (AD), a progressive and debilitating neurodegenerative disorder is one of the most common causes of dementia in the elderly and more than 40million people are living with AD worldwide (Francis *et al*., 1999; Khurana *et al*., 2021). The main aetiology of AD remains elusive; however, several hypotheses have been postulated to be involved in the pathophysiology of AD amongst which are Proteinopathy, lysosomal-mitophagy dysfunction, oxidative stress, and neuroinflammation (Tonnies and Trushina 2017; Bondi *et al*., 2017), and loss of the cholinergic innervation in the limbic and neocortical region of the brain (Hampel *et al*., 2018; Ishola *et al*., 2020). The cholinergic hypothesis has gained significant attention over the years and several studies have reported significant correlation between extensive depletion of cholinergic neurons in basal forebrain as well as reduced cholinergic fiber network of the cortical mantel and hippocampus and features of AD (Chen and Mobley 2019; Ishola *et al*., 2020; Khurana *et al*., 2021; Llanes *et al*., 2023), thus, resulting in

attention, learning and memory disability. Extracellular formation of senile plaques (comprising of aggregated amyloid-beta peptide (Aβ) and phosphorylated intracellular neurofibrillary tangles (Tau) are the main neuropathological indication of AD. Interestingly, increase in Aβ generation is directly correlated with intracellular neurofibrillary tangles formation, neuronal loss and synaptic dysfunction (Kamat *et al*., 2016).

Oxidative stress plays significant role in the etiopathogenesis of AD due to interaction of metal ions (copper, zinc or iron) with either Aβ peptide or tau peptide catalyze the production of reactive oxygen species (ROS) leading to oxidative damage of surrounding molecule including membrane lipid, protein or nucleic acid) (Tonnies and Trushina 2017; Ishola *et al*., 2017; Cheignon *et al*., 2018). Despite the increasing knowledge about the pathophysiology of AD, the disease condition still remains an unmet medical needs and burden to the healthcare system, economy and family globally. It is worthy of note that the current pharmacological interventions in the management of AD only proffer symptomatic relieve through enhancement of cholinergic neurotransmission and blockade of NMDA receptor (Ishola *et al*., 2019). ROS-induced oxidative damage in the hippocampus and neocortex are well linked with aging and AD development, thus, antioxidants could be a viable option in the management of AD, hence, slowing the progression of AD (Ishola *et al*., 2020). Interestingly, flavonoids are antioxidant and are very ubiquitous in plants with various health benefit including prevention of neurodegenerative diseases (Wan *et al*., 2019; Ishola *et al*., 2020).

In the present study, we evaluated the potential benefits of natural product rich in flavonoids, *Cajanus cajan* (L) Millsp, (Leguminosae) (pigeon pea) is majorly cultivated in tropical and semi tropical regions including Asia (especially south Asia), Africa and Latin America and serves as a major source of dietary protein. Interestingly, our preliminary quantitative chromatographic -spectroscopic analysis revealed the richness of *C. cajan* in flavonoids such as quercetin, cajanin, pinostrobin, cajaninstilbene, cajanolactone, formononetin, Biochanin A and B which is in agreement with previous studies (Hassan *et al*., 2015; Wu *et al*., 2019). Moreso, antioxidant, anti-inflammatory and antimicrobial activities of *C. cajan* have been reported (Zu *et al*., 2010; Hassan *et al*., 2015: Tekale *et al*., 2016). In traditional medicine, *C. cajan* is used in the treatment of neurological disorders, kidney diseases, diabetes, skin irritations, diarrhea, measles, and pain (Ahsan and Islam, 2009; Saxena *et al*., 2010; Pal *et al*., 2011). Hence, this study is designed to investigate the nootropic effect of ethanol seed extract of *C. cajan* (CC) on scopolamine-induced memory dysfunction in mice analogous to what is observed in patients living with dementia. Interestingly, scopolamine induced learning and memory processing impairments are reversed by acetylcholinesterase inhibitors (e.g. physostigmine). Amnesia caused by intraperitoneal injection of scopolamine is a common model of dementia in rodents suggestive of reduced cholinergic function (Ishola *et al*., 2019). Scopolamine, a muscarinic M1 receptor antagonist impairs cholinergic function and mitochondrial function (Klinkenberg and Blokland, 2010), leading to the generation of reactive oxygen radicals in the hippocampus, thus, leads to decline in memory and cognitive functions (Flood and Cherkin, 1986; Liao *et al*., 2020; Ishola *et al*., 2020). M1 Muscarinic acetylcholine receptors (mAChRs) are expressed in the hippocampus and cerebral cortex, where they play a significant role in the aberrant alterations of memory, cognitive processing, and learning, seen among people living with AD (Yousuf *et al*., 2023). Similarly, α7-nicotinic cholinergic receptors play pivotal role in memory modulation, thus, alteration in their function is linked with cognitive deficits. In this study, effort were made to assess the modulatory role of CC on M1 muscarinic acetylcholine receptors and α7- nicotinic cholinergic receptors activities using in silico techniques.

MATERIALS AND METHODS

Laboratory Animals: Adult mice of either sex used in this study (20-25g) were purchased from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed in plastic cages at room temperature and standard environmental conditions. The animals were fed with dried pellet (Livestock meal, Lagos, Nigeria) and clean water daily. The mice were allowed to acclimatize for a period of 7 days before the commencement of the experiment. The animals were properly handled and cared for in accordance with the Health Research and Ethics Committee (HREC) of the College of Medicine, University of Lagos, Nigeria with approval number (CMUL/ HREC/ 01/22/1000).

Drugs and Chemicals: Ethanol, donepezil hydrochloride, scopolamine hydrobromide (Sigma Aldrich St. Louis MO, USA), phosphate buffer 1x (Life Technology, USA).

Extract Preparation: Dried *Cajanus cajan* seeds were purchased from a local herb market in Lagos, Nigeria and pulverized to powder. The powder was then macerated in absolute ethanol for 72hours. It was filtered and filtrate was oven dried to yield a yellowish powder extract.

Experimental Procedure: Mice were randomly divided into seven groups (n=6) as follows; groups 1 and 2 received normal saline (10 ml/kg, p.o.), respectively, group 3-donepezil (1mg/kg; p.o.) and groups 4-7 received graded doses of *C. cajan* (50, 100 or 200mg/kg, p.o., respectively) for sixteen consecutive days. One-hour post-drug administration on day 3, mice in groups 2-6 were given scopolamine (1mg/kg, i.p.) from days 3 to 16 consecutively.

Open field Test (OFT): OFT is a protocol used to assay for locomotion activity, anxiety and readiness to explore in laboratory animals (Owope *et al*., 2016; Ishola *et al*., 2019). The OFT apparatus is a 96cm × 96cm × 45cm box made from wood. The floor of the apparatus is divided into 16 squares (18 × 18cm) by white lines. On day 11, each mouse was placed at the centre point of the apparatus and allowed to acclimatize for 60 seconds. Afterwards the total number of rearing, line crosses and grooming behaviour were recorded for 5mins. After each trial, the maze was cleaned with 10% ethanol and allowed to dry.

Y-Maze Test: The Y maze test is used to evaluate spontaneous exploration behaviour and short-term working memory (Ishola *et al*., 2020). The Y-maze is designed as a

Y shaped wooden apparatus with labelled arms A, B, C. After OFT on day 11, the animal was placed in the centre of the maze, and the total number of arm entries and spontaneous alternations defined as sequence of entries [ABC, BAC, CBA] were observed and recorded by an observer blinded to the treatment groups.

$$\% \text{ Spontaneous alternation} = \frac{\text{Number of alternation}}{\text{Number of entries - 2}} \times 100$$

Morris Water maze Task (MWM): MWM is designed to test spatial learning and memory ability of a rodent. The apparatus consist of a circular black tank (110cm diameter and 60cm height) to a depth of 30 cm filled with water up to 25cm high. The circular tank was divided into four hypothetical quadrants, designated as: N (North), E (East), W (West), S (South). A platform was placed 1.5cm beneath the water surface in the southwest quadrant. The mice were trained to locate the hidden platform within 60s and were allowed to stay on it for 10 s. The time taken for the mouse to locate the escape platform was recorded as escape latency (ELT). In the event that the animal was unable to locate the hidden platform within 60 s, it was gently guided to it and allowed to stay on it for 10 s. Three trials were conducted on each day for four days (days 12-15) (designated as spatial acquisition phase). On day 16, a probe test was carried out to assess retention memory, during which the escape/hidden platform was removed from the pool and the total time spent by the animal within the quadrant of the platform location was recorded within 30s (Ishola *et al*., 2013; Ishola *et al*., 2016; Ishola *et al*., 2019).

Dissection: After the probe test on day 16, the animals in each group were anaesthetized, then perfused with cold normal saline and brain was rapidly removed, and hippocampus was dissected on iced pack, weighed and kept in 0.1× PBS (pH 7.4) at −20°C until biochemical analysis.

Biochemical Analysis: Malondialdehyde (MDA) is a marker of lipid peroxidation, spectrophotometrically measured using the thiobarbituric acid assay procedure as previously described by Ishola *et al*. (2017). The reduced glutathione (GSH) content of brain tissue as non-protein sulphydryl was estimated according to the method described Sedlak and Lindsay (1968). The activity of superoxide dismutase (SOD) was assayed according to the method described by Nauseef *et al*. (2014). Similarly, we also estimated the nitrite level in mice brain using the Greiss reagent, which served as an indicator of nitric oxide production (Green *et al*., 2005). Catalase activity was also determined according to the method described by Sinha *et al*. (1972). The acetylcholinesterase (AChE) activity in the hippocampal homogenate was quantified using the protocol of Ellman *et al*. (1959).

Molecular Docking

Preparation of target protein: The Protein Data Bank (PDB) database was used to get the crystallographic structure of the target Ligand/receptor of interest (MI muscarinic acetylcholine receptor, α7 nicotinic acetylcholine receptor and amyloid beta42, (PDB ID: 6WJC, 3SQ9 and 3T4G respectively). We selected these structures because they have been used in other molecular docking studies (Li *et al*., 2011; Cheng *et al*., 2012; Maeda *et al*., 2020).

Ligand Preparation: For this study, the ligand structures were obtained from the PubChem database. Nineteen natural compounds identified to be present in *Cajanus cajan* seed from our preliminary study and standard reference drug for the target protein (Table 1).

Table 1:
Natural compounds earlier isolated from *Cajanus cajan* seed extract

S/N	Compound	PUBCHEM ID	IUPAC Name
1	Physcion	10639	1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione
2	Lupeol	259846	(1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol
3	Cajanol	442670	5-hydroxy-3-(4-hydroxy-2-methoxyphenyl)-7-methoxy-2,3-dihydrochromen-4-one
4	Quercetin	5280343	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
5	Biochanin A	5280373	5,7-dihydroxy-3-(4-methoxyphenyl)chromen-4-one
6	Formononetin	5280378	7-hydroxy-3-(4-methoxyphenyl)chromen-4-one
7	Vitexin	5280441	5,7-dihydroxy-2-(4-hydroxyphenyl)-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one
8	Apigenin	5280443	5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one
9	Luteolin	5280445	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one
10	Isoquercitrin	5280804	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one
11	Genistein	5280961	5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one
12	Isorhamnetin	5281654	3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromen-4-one
13	Orientin	5281675	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one
14	Pinostrobin	73201	(2S)-5-hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one
15	Friedelin	91472	(4R,4aS,6aS,6aS,6bR,8aR,12aR,14aS,14bS)-4,4a,6a,6b,8a,11,11,14a-octamethyl-2,4,5,6,6a,7,8,9,10,12,12a,13,14,14b-tetradecahydro-1H-picen-3-one
16	Naringenin	932	5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one
17	Cajanin	5281706	3-(2,4-dihydroxyphenyl)-5-hydroxy-7-methoxychromen-4-one
18	Daidzein	5281708	7-hydroxy-3-(4-hydroxyphenyl)chromen-4-one
19	Betulin	72326	(1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol
20	Donepezil	3152	2-(1-benzylpiperidin-4-yl)methyl-5,6-dimethoxy-2,3-dihydroinden-1-one

https://pubchem.ncbi.nlm.nih.gov/ *accessed October, 2022*

Table 2:
Showing the grid centers and Dimension of the binding pockets of each target protein

Target Protein	Grid Center	Dimension (Angstrom)
M1 Muscarinic ACh receptor	X- 21.1150, Y-27.7651, Z- 13.8030	X- 42.9568, Y- 40.4275, Z- 25.000
Alpha-2 nicotinic ACh receptor	X- 18.5315, Y-18.2748, Z-(-)13.1067	X- 38.7118, Y- 34.8079, Z- 25.0000
Amyloid beta	X- 17.4658, Y- 17.3212, Z- 2.7678	X- 25.0000, Y- 25.0000, Z- 25.0000

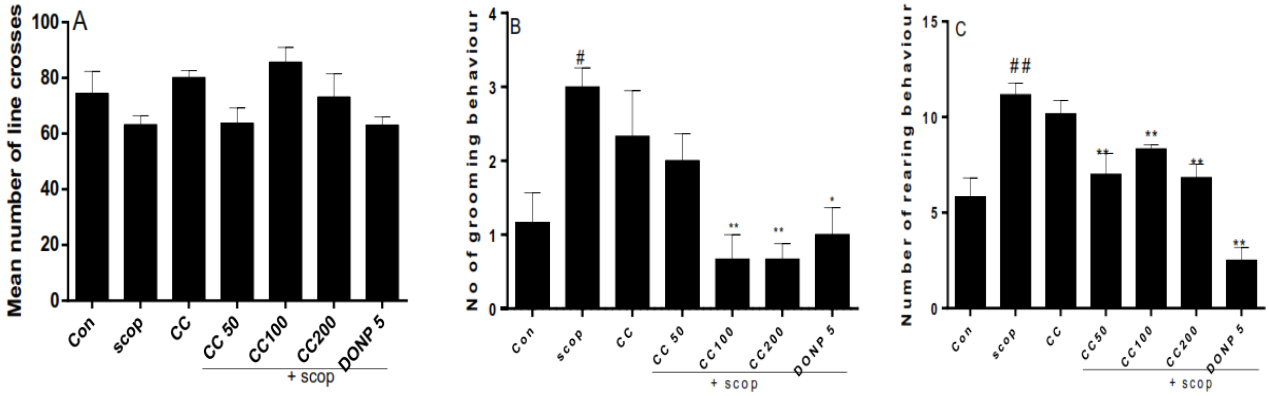

Figure 1 A-C:
Effect of CC and scopolamine on (A) number of line crosses, (B) number of rearing behaviour and (C) grooming behaviour in mice subjected to OFT. Values are expressed as mean±SEM (n=6). $^{*}p< 0.05$, $^{**}p<0.01$;$^{***}p<0.001$ versus scopolamine treated group and $^{\#}p<0.05$, $^{\#\#}p<0.01$ versus control treated group. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison test.

Docking study: The Biovia Discovery studio 3.0 and Pyrex software were used to conduct the studies on the target proteins. The Biovia discovery studio was used to prepare the protein for docking and post docking analysis while the Pyrex was used to generate binding sites (the box was placed in the catalytic region of the enzyme), the grid center and dimensions of the box in the X-, Y- and Z-axis was noted for the target protein (Table 2). The compounds were docked into the binding sites/pocket of the target protein using Pyrex and their receptor-ligand interactions determined using Biovia Discovery studio (Postdocking analysis).

Statistical Analysis: Data were expressed as mean ±SEM (n=6) and analyzed using one-or two-way ANOVA for repeated measures when appropriate, followed by Tukey post hoc multiple comparison test (whichever is applicable) using Graphpad prism version 6 (Graphpad prism Inc, CA, USA).

RESULTS

C. cajan **increases locomotor activities in open field test**: Administration of scopolamine and CC showed significant effects of treatment on number of line crosses [F(6,35) = 4.97, P< 0.001] (Fig. 1A), number of grooming [F(6,35)=5.595, P=0.0004] (Fig. 1B) and number of rearing behaviour [F(6,35)=14.46, P< 0.0001] (Fig. 1C). Administration of scopolamine and CC caused no significant change in number of line crosses compared to the control treated group. Conversely, scopolamine caused a significant increase in grooming behaviour relative to the control-treated group which was significantly reduced by the pretreatment of mice with CC 100 or 200mg/kg. Furthermore, oral administration of scopolamine increased rearing behaviour in comparison with control group. However, the pre-treatment of mice with CC failed to significantly reverse scopolamine-induced increase in rearing behaviour.

CC prevents scopolamine-induced working memory impairment in YMT: One way ANOVA showed significant effect of treatments on number of arm entries [F (6, 28) = 3.75, P < 0.007] (Fig. 2A) and percent spontaneous alternation [F (6, 28) = 10.46, P<0.0001] (Fig. 2B) in YMT. Post hoc multiple comparisons showed no significant change in mean number of arm entries among all the treatment groups when compared to the control treated group. However, the pretreatment of mice with scopolamine caused significant decrease in percent spontaneous alternation behaviour when compared with vehicle control. In contrast, CC 50 and 100mg/kg caused significant increase in spontaneous alternation behaviour when compared with scopolamine treated group (Figure 2B).

CC prevents scopolamine-induced spatial learning deficit in MWM: Two-way ANOVA showed significant effect of CC and scopolamine treatments on escape latency [F (6, 96) = 6.364, P < 0.0001] (Figure 3A) and probe trial [F (6, 25) = 8.044, P < 0.0001] (Figure. 3B) in MWM task. Scopolamine treated control produced no significant change in escape latency when compared with first session in the

spatial acquisition phase. However, CC pre-administration caused time course and significant decrease in escape latency when compared with first session in the spatial acquisition phase.

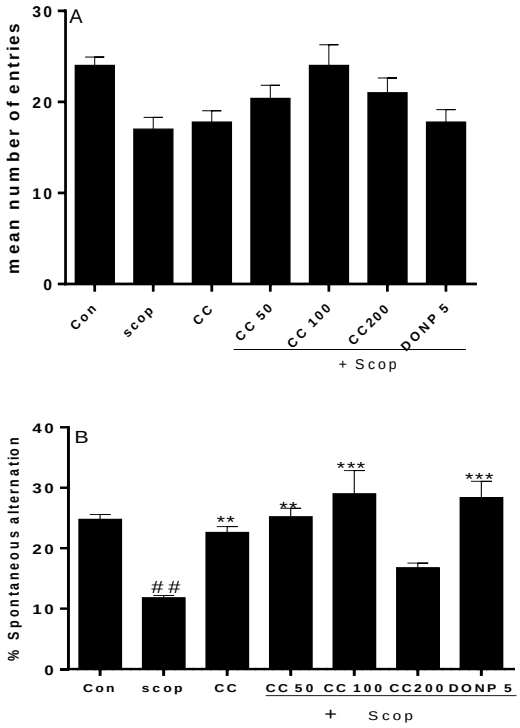

Figure 2A-B:
Effect of CC and scopolamine (A) number of arm entries and (B) percent spontaneous alternation behaviour in mice. Values are expressed as mean±SEM (n=5). $^{*}p< 0.05$, $^{**}p<0.01$,$^{***}p<0.001$ versus SCOP treated group; $^{\#\#}p<0.0001$ versus vehicle control treated group. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison test.

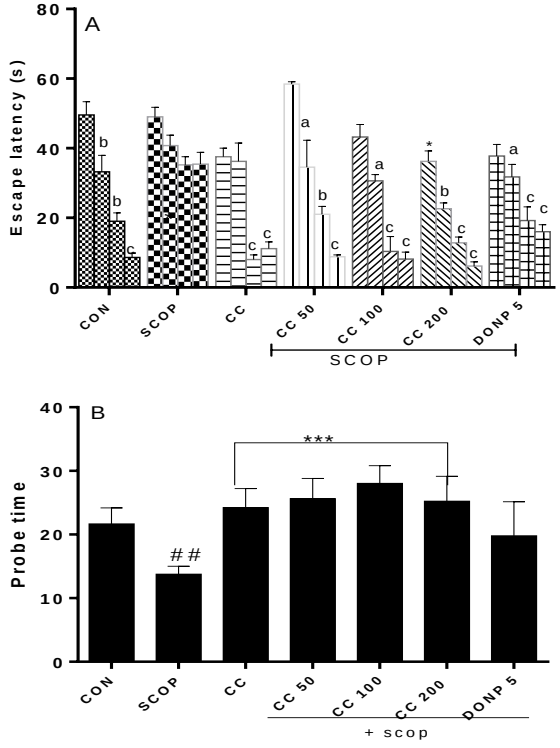

Figure 3A-B:
Effect of CC and scopolamine on (A) escape latency time and (B) probe trial in MWM task. Values are expressed as mean±SEM (n=5). $^{a}p< 0.05$, $^{b}p< 0.01$ $^{c}p< 0.001$ versus day 1., $^{\#\#}p<0.01$ versus vehicle control; $^{***}p<0.05$ versus SCOP-control treated group. Statistical level of significance analysis by one- or two-way ANOVA followed by Tukey *post hoc* multiple comparison test.

Moreso, in the probe trial, post hoc multiple comparison test showed significant reduction in time spent in the hidden platform location area by scopolamine treated when compared to the control group. Moreso, CC caused significant increase in time spent by the animal within the quadrant location when compared with scopolamine treated control.

CC attenuates scopolamine-induced MDA and nitrite generation: Scopolamine treatment caused significant increase in malondialdehyde (MDA) [F (6, 28) = 3.884, P = 0.0060], and nitrites [F (6,28)=6.308,P = 0.0003] generation in the hippocampus. Post hoc analysis showed that the pretreatment of mice with CC (50, 100 and 200mg/kg) significantly attenuated MDA and nitrite generation induced by scopolamine, with peak effect observed at CC 200mg/kg as shown in Figure 4A and B.

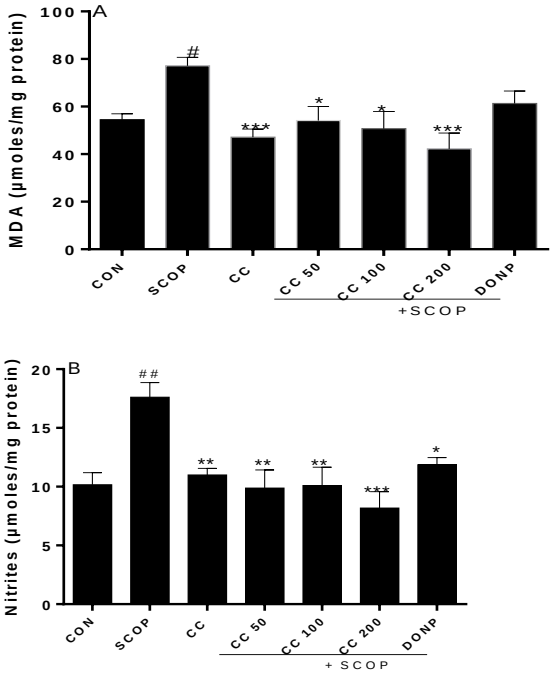

Figure 4 A-B:
Effect of CC and scopolamine (A) MDA and (B) nitrites. Values are expressed as mean ±SEM (n=5). $^{\#}P<0.05$, $^{\#\#}P< 0.01$versus vehicle-control treated group and $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, versus vehicle-scopolamine treated group. Statistical analysis was done using one way ANOVA followed by Dunnet multiple comparison test.

CC reversed scopolamine-induced hippocampal antioxidant enzymes deficits: One way ANOVA showed significant effect of subacute exposure to scopolamine and CC evidenced in a significant decrease in GSH level [F (6,35) = 13.06,P<0.001] (Fig. 5A), SOD [F (6, 35)=14.95, P < 0.0001] (Fig. 5B), and catalase [F (6, 28) = 17.51, P < 0.0001] (Fig. 5C) activities in the hippocampus. Tukey post hoc multiple comparison test showed that subacute administration of scopolamine significantly reduced GSH level (3.2 folds), SOD (2 folds) and catalase (1.5 folds)

when compared to normal control. However, the pretreatment of mice with CC 200mg/kg significantly reversed scopolamine-induced GSH level (3 folds), SOD (1.5 folds) and catalase (2 folds) when compared with scopolamine-vehicle treated group (Figure 5A-C). In another experiment, the administration of scopolamine significantly increased [F(6,28)=5.637,P=0.0006] acetylcholinesterase activity in the hippocampus relative to the control group. However, post hoc multiple comparison revealed that the pretreatment of mice with CC (100 or 200mg/kg) significantly inhibited this effect. Also, both CC 100 and 200mg/kg produced similar activity compared to the standard drug (donepezil) treated group as shown in Figure 5D.

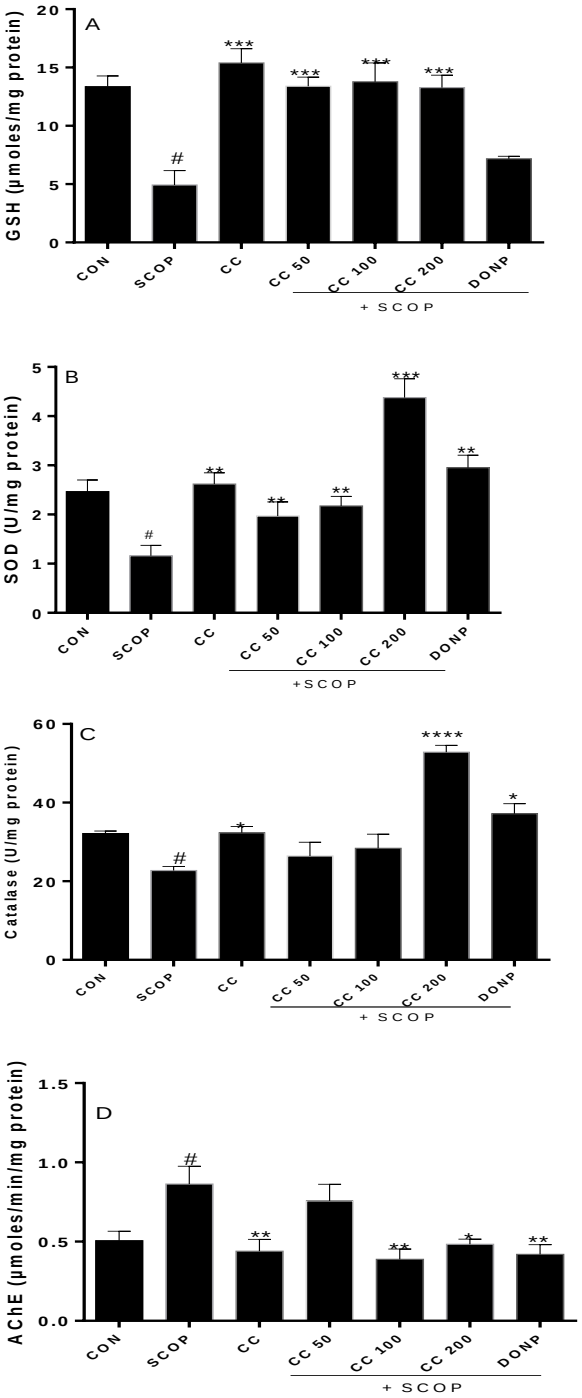

Figure 5A-D: Effect of CC and scopolamine on (A) GSH level, (B) SOD activity, (C) catalase activity and (D) acetylcholinesterase activity in the hippocampus. Values are expressed as Mean ±SEM (n=5). $^{\#}P<0.05$ versus vehicle-control treated group; $^{*}P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$ versus scopolamine treated group. Statistical analysis was done using One-way ANOVA followed by Tukey *post hoc* multiple comparison test.

Receptor-ligand Interactions

Receptor-ligand Interaction at Amyloid beta receptor site: Results from our molecular simulation showed that ligands such as friedeline (-7.5Kcal/mol), lupeol (-6.7Kcal/mol), apigenin and luteolin (-6.5Kcal/mol) binding affinity with amyloid beta active site compared with donepezil (acetylcholinesterase inhibitor, -5.8Kcal/mol) (Table 3). Also, these ligands interacted with amino acid residues: Lys9, Val7 and Leu5 while donepezil reacted with Ile2.

Table 3:
Binding Energy of ligands at Amyloid beta receptor site

S/N	Ligand	Chem ID	Binding Affinity ((Kcal/Mol)	rmsd /ub	rmsd /lb
1	Friedeline	91472	-7.5	0	0
2	Lupeol	259846	-6.7	0	0
3	Apigenin	5280443	-6.5	0	0
4	Luteolin	5280445	-6.5	0	0
5	Betulin	72326	-6.4	0	0
6	Physcion	10639	-6.3	0	0
7	Isorhamnetin	5281654	-6.3	0	0
9	Daidzein	5281708	-6.2	0	0
10	Biochanin A	5280373	-6.2	0	0
11	Pinostrobin	73201	-6.1	0	0
12	Cajanol	442670	-6	0	0
13	Naringenin	932	-6	0	0
14	Genistein	5280961	-5.9	0	0
15	Cajanin	5281706	-5.9	0	0
16	Quercetin	5280343	-5.9	0	0
17	Donepezil	3152	-5.8	0	0
18	Vitexin	5280441	-5.8	0	0
19	Orientin	5281675	-5.7	0	0
20	Formononetin	5280378	-5.7	0	0
21	Isoquercitrin	5280804	-5.6	0	0

Analysis of the mode of docking of the ligands at M1 Muscarinic ACh receptor: Results obtained from the docking study showed that pinostrobin (-8.7Kcal/mol), friedeline (-8.3Kcal/mol), formononetin and vitexin (-7.6Kcal/mol) binding affinity with the M1 muscarinic ACh active site with binding affinity better than donepezil (standard acetylcholinesterase inhibitor) as shown in Table 4. Showed good interaction with Pro97, Leu89, Ala93, Trp145 via pi-pi alkyl bond.

Analysis of the mode of docking of the ligands at alpha-7 nicotinic receptor: Lupeol (-8.2Kcal/mol), friedeline (-8.1Kcal/mol), botulin (-7.8Kcal/mol), orientin and vitexin (-7.6Kcal/mol) binding energy which was than that of the standard drug, donepezil (-7.2Kcal/mol) at the alpha-7 nicotinic acetylcholine binding pocket (Table 5). Post docking analysis showed that the compounds with better binding affinity interacted with Tyr115, Gln121 and Ala90

via hydrogen bond and Pro97 through alkyl bond. Other interactions were also formed with Ala89, leu88 amino acid residues.

Table 4:
Binding Energy of ligands at M_1 muscarinic ACh receptor site

S/N	Ligand	Chem ID	Binding Affinity (Kcal/mol)	rmsd/ ub	rmsd/ lb
1	Pinostrobin	73201	-8.7	0	0
2	Friedeline	91472	-8.3	0	0
3	Formononet	5280378	-7.6	0	0
4	Vitexin	5280441	-7.6	0	0
5	Apigenin	5280443	-7.6	0	0
6	Isorhamnetin	5281654	-7.5	0	0
7	Cajanin	5281706	-7.5	0	0
8	Daidzein	5281708	-7.5	0	0
9	Biochanin A	5280373	-7.4	0	0
10	Luteolin	5280445	-7.4	0	0
11	Genistein	5280961	-7.3	0	0
12	Physcion	10639	-7.2	0	0
13	Quercetin	5280343	-7.1	0	0
14	Naringenin	932	-7	0	0
15	Donepezil	3152	-7	0	0
16	Isoquercitrin	5280804	-7	0	0
17	Cajanol	442670	-6.8	0	0
18	Betulin	72326	-6.6	0	0
19	Orientin	5281675	-6.1	0	0
20	Muscarine	9308	-5.2	0	0

Table 5:
Binding Energy of ligands at alpha-7 nicotinic receptor site.

/N	Ligand	Chem ID	Binding Affinity (Kcal/mol)	rmsd /ub	rmsd /lb
1	Lupeol	259846	-8.2	0	0
2	Friedelin	91472	-8.1	0	0
3	Betulin	72326	-7.8	0	0
4	Orientin	5281675	-7.6	0	0
5	Vitexin	5280441	-7.3	0	0
6	Isoquercitrin	5280804	-7.2	0	0
7	Donepezil	3152	-7.2	0	0
8	Cajanin	5281706	-6.9	0	0
9	Pinostrobin	73201	-6.9	0	0
10	Physcion	10639	-6.8	0	0
11	Cajanol	442670	-6.8	0	0
12	Quercetin	5280343	-6.8	0	0
13	Isorhamnetin	5281654	-6.8	0	0
14	Luteolin	5280445	-6.7	0	0
15	Genistein	5280961	-6.7	0	0
16	Daidzein	5281708	-6.7	0	0
17	Naringenin	932	-6.6	0	0
18	Apigenin	5280443	-6.4	0	0
19	Biochanin A	5280373	-6.1	0	0
20	Formononeti	5280378	-5.7	0	0

DISCUSSION

Findings from this study revealed that scopolamine caused spatial learning and memory deficits as well as cholinergic dysfunction and oxidative stress in the hippocampus (Ishola *et al*., 2020; Liao *et al*., 2020), however, *C. cajan* seed extract attenuated scopolamine-induced cognition, antioxidant system and cholinergic neurotransmission deficits in mice. Moreso, *C. cajan* phytochemicals such as friedeline, Pinostrobin and lupeol showed significant interaction with neuronal M1 muscarinic cholinergic receptor, α7 nicotinic cholinergic receptor and amyloid-beta peptide indicative of their ability to improve cholinergic signaling as well as preventing amyloid-beta aggregation. Several reports have shown that acetylcholine plays pivotal role in the regulation of adult hippocampal neurogenesis (implicated in learning and memory), as the dentate gyrus receives afferent cholinergic inputs from the basal forebrain (Li *et al*., 2022). Moreso, the current treatment option for AD involves synaptic elevation of acetylcholine level through inhibition of acetylcholinesterase enzymes activity. Interestingly, inhibitors of acetylcholinesterase enzymes activity promote neurogenesis. In addition, acetylcholine signaling has been shown to strengthen the associations between environmental cues and reward availability, thus, improves learning and memory (Ishola *et al*., 2017).

Scopolamine, a muscarinic receptor antagonist, widely used to model cognitive decline in rodents as seen in AD (Ishola *et al*., 2020). Salimi *et al*. (2022) posited that scopolamine-induced memory and learning impairment is associated with mitochondrial dysfunction, neuroinflammation and oxidative stress which have also been linked with AD pathology. In this study, we evaluated the effect of scopolamine on spatial recognition memory in Y-maze (Ishola *et al*., 2016). Y-maze is a behavioural test used to investigate spatial recognition as well as spontaneous alternation behaviour in rodents (Ishola *et al*., 2020). Animals treated with scopolamine had increased spontaneous motor activity with no significant change in spontaneous alternation. In the present study, scopolamine reduced percent spontaneous alternation movement suggestive working memory impairment. In contrast, the pretreatment of mice with CC caused significant improvement in percent alternation behaviour indicative of enhanced spatial memory (Ishola *et al*., 2020). To ascertain the impact of CC on acquisition spatial learning and retention memory, the MWM task was carried out.

The Morris water maze test is a common behavioural model used to evaluate spatial learning and memory function (Saba *et al*., 2017; Lee *et al*., 2018; Ishola *et al*., 2020). In this study, mice in the control group quickly acquired spatial learning evidenced by the decrease in time course of escape latency following the different acquisition spatial learning trials and an increased time spent in the escape target quadrant location during the probe test. Similar to previous studies, scopolamine treated group showed an increased time swimming and lesser time in the escape target quadrant in the probe test indicative of spatial learning and retentive memory deficits (Saba *et al*., 2017; Lee *et al*., 2018; Ishola *et al*., 2020; Khurana, *et al*., 2021). However, pretreatment of animals with CC showed time course decrease in latency and increased time spent quadrant of hidden platform location which depict improvement in hippocampal spatial learning and retentive memory (Ishola *et al*., 2020).

Literature findings have reported that oxidative stress plays a key role in the pathogenesis and exacerbation of neurodegenerative disorders like AD and it is characterized

by increased production of reactive oxygen/nitrogen species (ROS/RNS) leading to decreased superoxide dismutase, and glutathione level, with a concomitant increase in malondialdehyde production (Chen and Zhong, 2014; Souza Ferreira *et al*., 2015; Tsikas, 2017). In AD, oxidative stress disrupts synaptic activity and neuronal signaling resulting in memory impairment and it has also been linked with mitochondrial dysfunction as well as accumulation of amyloid beta (hallmark of AD) which could worsen the disease prognosis (Tönnies and Trushina, 2017; Ishola 2019). In this present study, we observed a significant decrease in antioxidant enzymes; GSH, SOD and catalase activities as well as significant increase in hippocampal MDA and nitrite levels following subacute exposure of mice to scopolamine indicative of oxidative and nitrosative stress. However, the pretreatment of mice with CC reversed scopolamine-induced lipid peroxidation and nitrative stress through significant increase in antioxidant enzymes activity in the hippocampus supporting the earlier reported antioxidant properties of CC (Hassan *et al*., 2015).

In another experiment, the effect of CC on scopolamine-induced acetylcholine hydrolysis was investigated. It is well known that an increase in acetylcholinesterase activity would invariably result in acetylcholine hydrolysis into choline and acetic acid leading to loss or reduced cholinergic function. The central cholinergic system contributes to learning and memory functions. Moreso, acetylcholine is one of the important neurotransmitters that plays a critical role in regulating cognitive performances as well as learning and memory processes (Haam and Yakel, 2015; Ishola *et al*., 2020; Liao *et al*., 2020). In line with previous studies, scopolamine-induced an increase in acetylcholinesterase activities in the hippocampus of mice (Ishola *et al*., 2017; 2020). However, scopolamine induced increase in acetylcholinesterase activity was inhibited by CC subacute administration which further lend credence to the ability of CCt to enhance hippocampal cholinergic signaling.

To further validate the effect of *C. cajan* on cognition, molecular docking simulations were used to explore the potential interaction of the secondary metabolites with cholinergic receptors and amyloid-beta peptides. Our earlier reported compounds present in *C. cajan* were docked with M1 muscarinic acetylcholine receptor, alpha-7 nicotinic cholinergic receptor and amyloid beta. Muscarinic acetylcholine receptors plays distinct roles in the regulation of learning and memory processes, such as encoding cue-reward associations and consolidating these associations in long-term memory. Similarly, the α7 nicotinic acetylcholine receptors are highly ubiquitous in the hippocampus cortex where they play a pivotal role in memory formation, as such considered a potential therapeutic agents target (Jerusalinsky *et al*., 2000; Servent *et al*., 2011). Lastly, amyloid-beta is one of the key molecules in the pathogenesis of AD. Our results showed that phytochemicals from *C. cajan* seed had favorable receptor-ligand complex via pi-alkyl bonds with Pro97, Ala93, Leu89, Pro117 and Trp 145 similar to the interactions formed by donepezil, a cholinesterase inhibitor. Docking results also showed that phytocompounds from CC formed favourable receptor-ligand complex with alpha-7 nicotinic receptor, indicative of significant role for neuronal nicotinic cholinergic signaling. In silico study also showed that phytochemicals present in CC including Pinostrobin, friedeline, formononetin and vitexin showed better binding affinity for M1 muscarinic acetylcholine receptor active sites indicative of the involvement of M1 muscarinic ACh in cognition enhancing-like activity of CC. in addition, several reports of neuroprotective and cognitive enhancing action of formononetin, vitexin and friedeline have been reported (Fei *et al*., 2018; Fu *et al*., 2019; Sandhu *et al*., 2022). It is well known that M1 muscarinic acetylcholine receptors are largely found in the hippocampus and is said to have precognitive effects (Green *et al*., 2000; Zhao *et al*., 2018). Moreso, molecular docking simulation of the natural compounds with amyloid beta sheet revealed better interaction with the binding site/pocket of the target protein indicative of their ability to reverse amyloid-beta aggregation formation. Previous study has also shown the ability of friedelin to improve neuronal synapse and reversed scopolamine-induced memory impairment through inhibition of β-secretase enzyme (BACE-1) to halt amyloidogenic pathways of amyloid-β production (Sandhu *et al*., 2022).

In conclusion, our observations from this study showed that C.cajan seed extract prevents scopolamine-induced learning and memory deficits, oxidative stress and cholinergic deficit through enhancement of antioxidant defense mechanisms and cholinergic signaling.

Acknowledgement

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Full length Research Article

Antioxidant Activity Enhancement and Oxidative Damage Inhibition by *Lagenaria breviflora* fruit and *Xanthosoma sagittifolium* corm in Hypertensive Wistar Rats

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Summary: Cardiovascular diseases are the leading causes of mortality in the world today with hypertension being the major clinical presentation of these diseases. This study assessed anti-hypertensive effects of *Lagenaria breviflora* whole fruit and *Xanthosoma sagittifolium* corms in experimentally-induced hypertensive Wistar rats. The ability of the plants to ameliorate oxidative damage accompanying hypertension was evaluated using changes in oxidative stress markers as well as monitoring of cardiovascular parameters. Hypertension was induced by intraperitoneal injection of DOCA salt twice weekly and daily inclusion of NaCl (1%) in drinking water. Methanol extracts of *L. breviflora* or *X. sagittifolium* was administered to hypertensive rats for 35days and the outcome was compared to hypertensive rats administered with lisinopril or hydrochlorothiazide and a group of normotensive rats (control). Systolic, diastolic and mean arterial pressures were determined on day 34 and blood sample collected on day 35. The rats were thereafter humanely sacrificed and organs were harvested. This study showed the extracts lowered blood pressure, free protein thiols but increased total proteins, glutathione peroxidase, reduced glutathione, glutathione S-transferase, catalase and nitric oxide in the heart, kidney and liver compared to untreated hypertensive rats. However, malondialdehyde levels and hydrogen peroxide activities were reduced. *L. breviflora* fruit and *X. sagittifolium* corm exhibited antihypertensive properties and ameliorated oxidative damage associated with hypertension by enhancing the antioxidant defense system and inhibiting generation of free radicals.

Keywords*: Hypertension, Oxidative stress, Antioxidant, Lagenaria breviflora , Xanthosoma sagittifolium*

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INTRODUCTION

Hypertension is a medical condition clinically determined as systolic values ≥140 mmHg and diastolic ≥90 mmHg, with normotensive values averagely at 120/80mmHg (WHO, 2011). Arterial hypertension is characterized by increased oxidative stress and inflammation, which are associated with further cardiovascular risk. An increase in oxidative stress favors endothelial dysfunction by reducing nitric oxide (NO) availability and subsequent beneficial effects on vascular function (Ghosh *et al*., 2004; Pierini and Bryan, 2015). Oxidative stress is sequel to a shift in the normal equilibrium of oxidant molecules in the body to the anti-oxidants which results in generation of excessive reactive oxygen species (ROS) clinically presented as oxidative stress (Puddu *et al*., 2009; Pohanka *et al*., 2017). Amelioration of oxidative stress with pharmacological doses of different antioxidants has proven to reduce blood pressure in hypertensive animals, but not in the normotensive subjects (Ding *et al*., 2001; Koo and Vaziri, 2003; Rodriguez-Iturbe *et al*., 2003).

Important occurrence in the hypertension pathogenesis include increased cardiac output and total peripheral resistance. The kidneys control blood pressure through regulation of blood volume (Berl and Henrich, 2006). A close association has been established between hypertension and progressive kidney dysfunction, manifested as glomerulosclerosis, interstitial fibrosis, proteinuria, and eventual glomerular filtration decline (Tedla *et al*., 2011). Thus, treatment of hypertension has been geared towards enhancement of renal function and reduction of oxidative stress induction. Antioxidants play a key role in removal of free radical intermediates and inhibition of oxidation reactions caused by reactive oxygen species generated from oxidative stress. Medicinal plants with antioxidant properties are proving to be good alternative antihypertensives with lesser adverse effects compared to orthodox synthetic drugs (Tabassum and Ahmad, 2011; Eghianruwa *et al*., 2016).

Lagenaria breviflora , a perennial climbing plant belonging to the family Cucurbitaceae is used traditionally in the treatment of diseases of inflammatory origin such as diabetes, ulcer, jaundice, piles, colitis, congestive cardiac failure and skin disease (Oridupa and Saba, 2012). *L. breviflora* has antibacterial activity (Tomori *et al*., 2007), anti-inflammatory, anti-nociceptive and antioxidant properties (Oridupa and Saba, 2012; Onasanwo *et al*., 2011). Xanthosoma sagittifolium, also known as arrow leaf elephant's ear is an edible starchy corm in the family Araceae (Jennings, 1987). It is reported to exhibit potent antihyperglycemic effect (Folasire *et al*., 2016). The use of herbal remedies is effective with chronic conditions such as hypertension and are cheaper with less side effects when compared to orthodox drugs. There is currently a dearth of information on the antihypertensive effects of these medicinal plants. This study was therefore designed to investigate the antihypertensive properties of *L. breviflora* and *X. sagittifolium* on experimentally-induced hypertension in Wistar rats and the effect of these plants on oxidative stress which accompanies hypertension.

MATERIALS AND METHODS

Preparation of methanol fruit extract: Fresh fruit of *Lagenaria breviflora* Roberty and corms of *Xanthosoma sagittifolium* Schott were obtained from the Agbowo and Oje Markets in Ibadan, Oyo State, Nigeria. The plant samples were identified by a botanist in Botany Department, University of Ibadan. A total of 5kg of fresh fruit were washed and cut into small pieces and dried with a hot air oven at a temperature of 25ºC. The bark of the corms was peeled and cut into smaller pieces and were air dried. A total of 525g was obtained from the corms.

Extraction of the plant samples: The whole fruit or corms were separately tied up in small portions in sieves and extracted by cold maceration in methanol (96%) in well labelled containers. Each batch of solvent were harvested and stored in plastic containers. The filtrate obtained were concentrated in vacuo using rotary evaporator (BUCHI R-210, Switzerland) set at low temperatures. The methanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperature of 30ºC. The extracts obtained were refrigerated at 40C and fresh batches of the extracts were reconstituted with distilled water daily for administration to the rats.

Antihypertension Study: Forty normotensive male Wistar rats (150-180g) were used for the study. The rats were housed at the Experimental Animal House of the Department of Veterinary Pharmacology & Toxicology, University of Ibadan. They were allowed access to feed (commercially available rat pellets) and clean water ad libitum. The experiment was conducted in accordance with Experimental Animal Care and Use Regulations Ethics Committee (ACUREC) of the University of Ibadan, Nigeria which is acceptable internationally.

The rats were randomly divided into eight groups of five rats each. Group one served as the normotensive control while hypertension was induced in groups 2 – 8 by intra-peritoneal injection of deoxycorticosterone acetate (DOCA) salt twice weekly and daily inclusion of 1% sodium chloride (NaCl) in water. The hypertensive rats were fed with commercially available rat pellets and NaCl (1%) in drinking water daily. Group 2 were hypertensive and untreated through the course of the study and groups 3 and 4 were orally administered with Lisinopril 5mg/70kg or Hydrochlorothiazide 12.5mg/70kg body weight respectively. Groups 5, 6, 7 and 8 were administered with 100 or 200mg/kg of the whole fruit extract of *L. breviflora* Roberty or corm extract of *X. sagittifolium* (L) Schott respectively. This study was conducted for the period of 35 days for treatment of hypertension after induction of hypertension.

Sample Collection: Cardiovascular parameters (mean arterial pressure, systolic and diastolic blood pressures) were measured on day 34 by non-invasive tail plethysmography method without anaesthesia using an electro-sphygmomanometer (CODA, Kent Scientific, USA). On day 35, blood samples (3ml) were collected into lithium heparinized bottles from the retro-orbital sinus of each rat for determination of markers of oxidative stress. Serum was obtained by centrifugation of blood for 10minutes and at 3000rpm and the serum was stored at -20 ºC until use.

The rats were thereafter humanely sacrificed and the heart, liver and kidney of each rat was carefully removed, perfused immediately with normal saline and blotted with filter paper. It was homogenized in cold potassium phosphate buffer (0.1 M, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 10000 rpm for 10 minutes with a cold centrifuge at 4 0C to obtain post mitochondrial fraction (PMF). The supernatant was used as the sample for determination of markers of oxidative stress assay which estimated levels of total protein, protein thiol, reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), malondialdehyde (MDA), catalase, hydrogen peroxide (H2O2) and nitric oxide (NO). The biochemical assays were determined by standard protocols according to the methods described by Olaleye *et al*. (2007).

Statistical analysis: Data on biochemical analysis were reported as mean ± standard deviation and were analyzed using ANOVA and subsequently Tukey Kramer multiple comparison test in GraphPad Prism, version 5.0 0 (Graph Pad Software, San Diego, CA, USA) and values of $p< 0.05$ were considered significant.

RESULTS

Systolic, diastolic and mean arterial pressures were significantly reduced by the higher doses (200mg/kg) of the extracts compared to the untreated hypertensive rats, especially by *X. sagittifolium* which was statistically unchanged compared to the control rats. Total protein levels in the liver and kidney of hypertensive untreated rats (5.45±0.56mg/dl and 2.85±0.20mg/dl) were significantly lower than that of the normotensive control (9.68±0.75mg/dl and 4.18±0.21mg/dl). Although total proteins in test animals were lower than the control rats, the

levels were significantly higher than those of the hypertensive untreated rats.

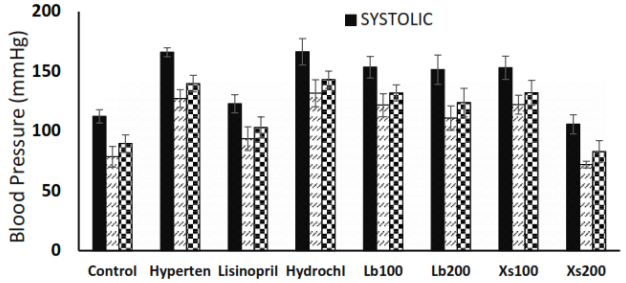

Figure 1:
Blood pressure of hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

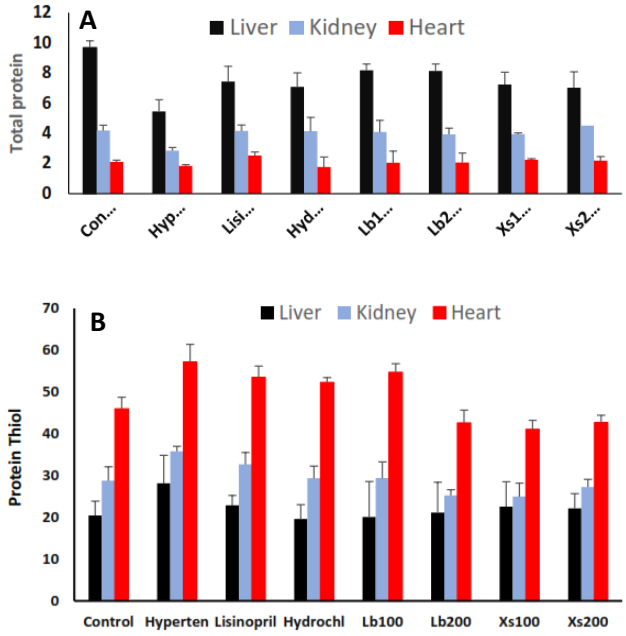

Figure 2:
Serum protein (A) and protein thiol (B) levels of hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

Total protein levels in the heart, although reduced, were not significantly changed when the test rats were compared to the control rats. Protein thiol levels were significantly ($p<0.05$) increased in all the organs, particularly in the kidney and heart of hypertensive untreated rats (28.13±1.22 µmol/L, 35.79±1.71 and 57.20±2.61 µmol/L) compared to the control rats (20.44±2.31, 28.77±2.77 and 45.98±1.67 µmol/L). Rats treated with *L. breviflora* (200mg/kg) or *X. sagittifolium* (100 or 200mg/kg) extracts had protein thiol levels that were statistically unchanged compared to the control rats.

The result showed that glutathione peroxidase (GPx) expression was statistically unchanged in liver, but the kidney and heart of hypertensive untreated rats showed significant ($p<0.05$) decline compared to the controls rats. This decline was reversed in hypertensive rats with the *L. breviflora* or *X. sagittifolium* extracts. *L. breviflora* (200mg/kg) showed significant increase ($p<0.05$) in GPx expression in the heart. Hypertensive untreated rats showed the least levels of glutathione expression (GSH) in all the organs with marked decline in GSH levels from the liver. Slight increases in the hypertensive rats treated with the extracts were however observed compared with the untreated rats. Glutathione-S-Transferase (GST) levels were significantly ($p<0.05$) decreased in the hypertensive untreated rats, but markedly high levels were observed in rats administered with the extracts, especially in the liver of rats administered with *X. sagittifolium* at 100mg/kg (151.55±15.44).

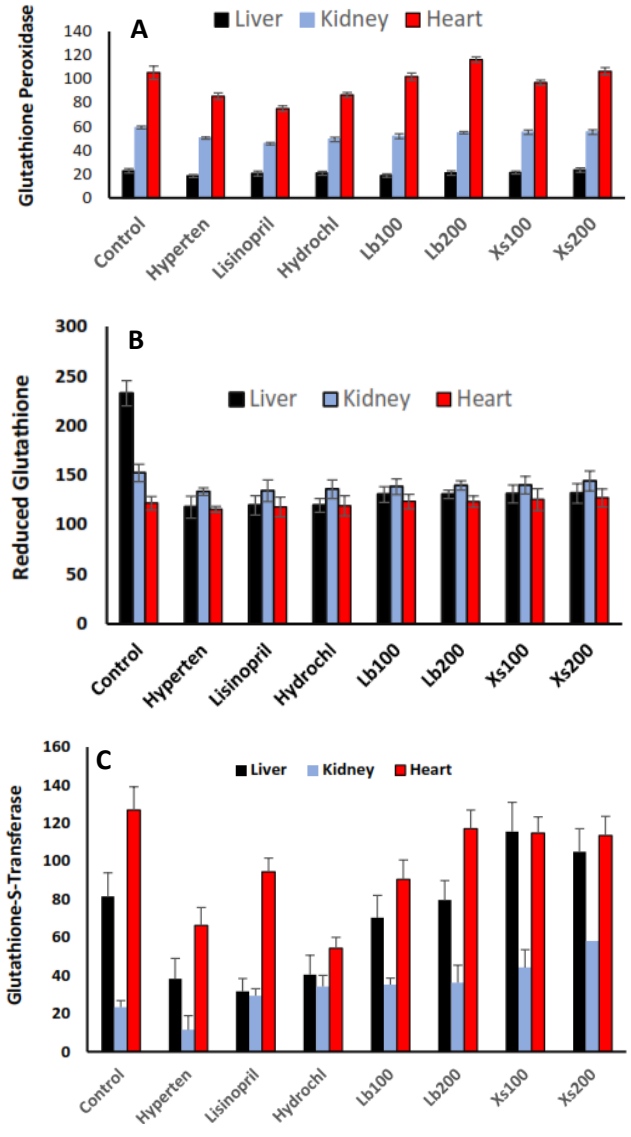

Figure 3:
Glutathione peroxidase (A), reduced glutathione (B) and glutathione –S-transferase (C) levels in hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

Malondialdehyde (MDA) levels in the organs of hypertensive untreated rats were significantly ($p<0.05$) higher compared to rats treated with the extracts, particularly in the liver. MDA levels in extract treated rats were comparable to that observed in control rats. Rats treated with *X. sagittifolium* extract showed the most consistently comparable levels to the control rats. Catalase levels in the liver and kidneys of all hypertensive rats were reduced, but a non-significant increase was observed in

heart of rats administered with the *L. breviflora* (100 and 200mg/kg) or *X. sagittifolium* (100mg/kg) extracts.

Hydrogen peroxide (H_2O_2) levels in the liver of hypertensive rats treated with *L. breviflora* were significantly ($p<0.05$) increased compared to normotensive rats. Although, the levels in liver of hypertensive rats treated with *X. sagittifolium* were increased, it was non-significantly lower than that observed in untreated hypertensive rats. Levels of H_2O_2 in the kidneys of all treated rats were non-significantly lower than that of control rats but significantly ($p<0.05$) lower than in untreated hypertensive rats. The heart of treated hypertensive rats showed varying levels of non-significant ($p>0.05$) reductions in the level of H_2O_2 compared to untreated hypertensive rats.

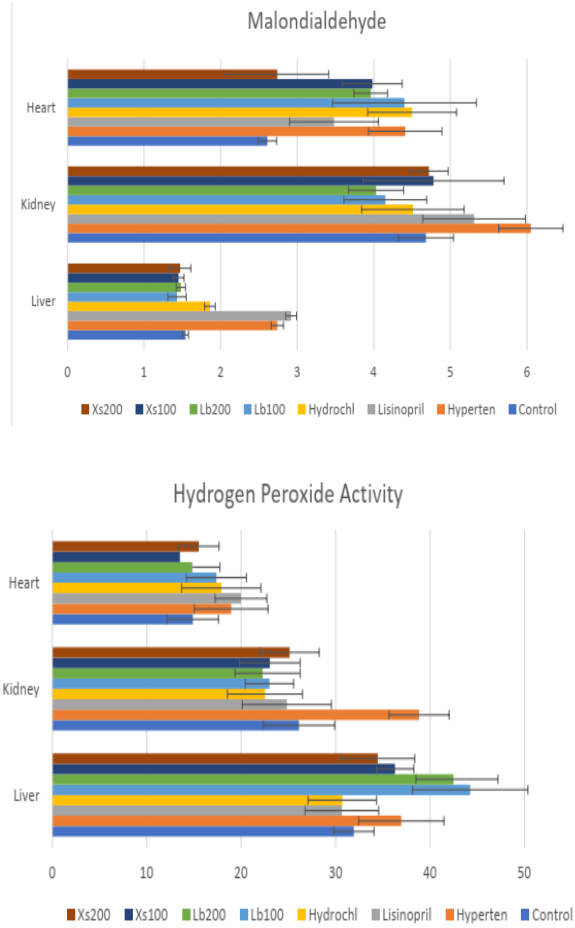

Figure 4:
Malondialdehyde and hydrogen peroxide levels in hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

Liver of hypertensive rats treated with *L. breviflora* had significantly higher levels of nitric oxide compared to that of control rats while those treated with *X. sagittifolium* was statistically unchanged. Nitric oxide level in the liver was highest in hydrochlorothiazide group and least in the hypertensive control.

DISCUSSION

This study evaluated the antihypertensive effect of methanol extracts of *Lagenaria breviflora* whole fruit and *Xanthosoma sagittifolium* corm in experimentally induced hypertensive rats. The result showed the higher doses of the extracts lowered the blood pressure, particularly X. sagittifolium. The main target of management of hypertension is blood pressure control (Rosei, 2016) which these plants gave promising results warranting further study on the exact mechanism involved. A major complication of hypertension, clinically presented as oedema in some hypertensive patients is hypoalbuminaemia (Blankfield *et al*., 2000). The significantly low protein levels seen in the liver, kidneys and heart of hypertensive untreated rats was reversed in the plant extracts treated hypertensive rats and this was comparable to the hypertensive rats treated with lisinopril, an ACE inhibitor (Khan. 2015) and hydrochlorothiazide, a thiazide diuretic (Vongpatanasin, 2015).

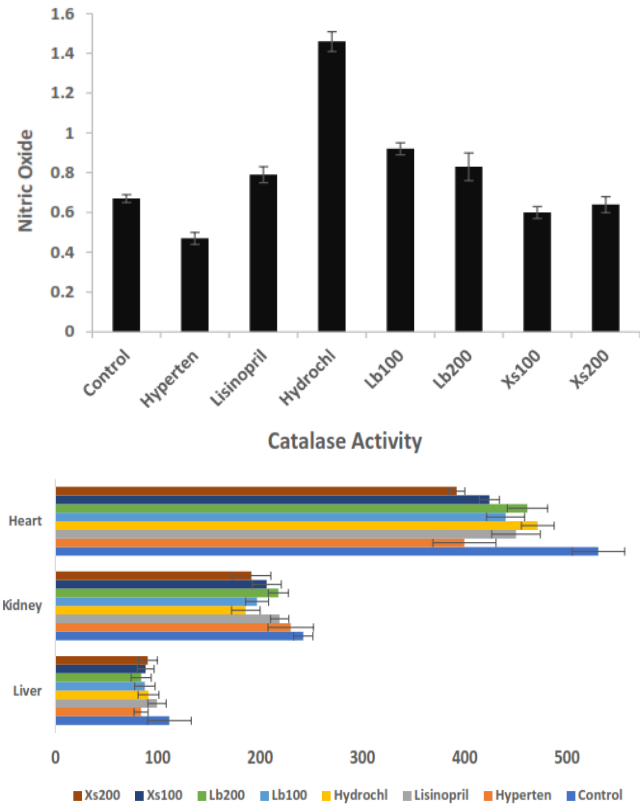

Figure 5:
Nitric oxide levels and catalase activity in hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

In this study, hypertension was experimentally induced by bi-weekly intraperitoneal injection of DOCA salt and oral loading with 1% sodium chloride. High sodium diet is associated with increased intra renal angiotensin II (Chen *et al*., 2020) which may result in renal vasoconstriction and increased renal O2- production due to activation of NADPH oxidase. Over production of superoxide anions and other free radicals due to activation of NADPH oxidase may overwhelm the antioxidant capability and cause imbalances between oxidant and antioxidant status which result in oxidative stress (Muhammad *et al*., 2012). The production of reactive oxygen species (oxidative stress) is the important etiological factor in the development of various diseases

including hypertension (Barrows *et al*., 2019; Verma *et al*., 2019).

A major antioxidant defense against free radicals is protein thiol (Birben *et al*., 2012). Most protein thiols occur bound to albumin in the blood, forming bonds with the sulfhydryl group at its cysteine-34 portion (Rossi *et al*. 2008). Albumin maintains colloid osmotic pressure in the blood and a shift in the balance such as seen in hypoalbuminaemia, clinically presents as excess circulating thiols (Toyoda *et al*., 2020). Increase protein thiol levels in untreated hypertensive rats further showed hypoalbuminaemia, with more unbound thiols in circulation. This was reversed in hypertensive rats treated with the extracts of *L. breviflora* or *X. sagittifolium* and were comparable to that observed in normotensive rats. Thiols have other roles separately from their role in defense against free radicals and this include their role in signal transduction, detoxification, apoptosis and various other functions at the molecular level (Brown and Griendling, 2015). Protein thiol has also been associated with diabetes mellitus, alcoholic cirrhosis, disorders related to kidney particularly chronic renal failure, cardiovascular disorders such as stroke and several neurological disorders (Prakash *et al*., 2009).

Glutathione, another form of thiol which occurs in the free oxidized or reduced state, is a known antioxidant in plants and animals which prevents cellular damage caused by reactive oxygen species to important cellular component (Pompella *et al*., 2003). Glutathione serves as an electron donor to reduce disulfide bonds formed within cytoplasmic proteins to cysteines. This process converts glutathione to its oxidized form; glutathione disulfide (GSSG). GSH act directly as an antioxidant or a cofactor for several protective enzymes important for cellular defense against oxidative stress (Lushchak, 2012; Koohpeyma *et al*., 2020). In this study, GSH levels were statistically unchanged in all hypertensive rats. However, extract treated rats had significantly increased glutathione peroxidase and glutathione-s-transferase levels. Glutathione peroxidases are a family of peroxidases that protect the body from oxidative damage by reducing lipids and hydrogen peroxides (Espinoza *et al*., 2008), while glutathione-s-transferases, a family of phase II detoxification enzymes, catalyze GSH conjugation to a wide assortment of endogenous and exogenous electrophilic compounds (Townsend and Tew, 2003).

Another antioxidant is nitric oxide which was restored to slightly above normal levels by the extract in this study. Nitric oxide protects against increased blood pressure and cardiac hypertrophy which are very key in the hypertensive state (Liu *et al*., 2005). The nature of nitric oxide as a messenger molecule is unusual with many physiologic roles, in many systems including the cardiovascular, neurologic and immune systems. Nitric oxide is known to mediate pathogen suppression, blood vessel relaxation and neurotransmission (Toda and Okamura. 2003). Nitric oxide in excessive amounts may cause host cell injury triggering neurotoxicity during strokes, and initiating hypotension related to sepsis (Madan and Rao. 1996). Furthermore, this study showed catalase activity increased significantly particularly in the heart of extract treated rats compared to the decline in untreated hypertensive rats. Catalase is an enzyme which occur in approximately all living organisms with oxygen exposure. This enzyme catalyzes hydrogen peroxide breakdown to water and oxygen, an important protective mechanism against ROS-induced oxidative damage (Chelikani *et al*., 2004).

This study also showed significant reduction in malondialdehyde and hydrogen peroxide, major markers of oxidative damage. MDA is the main product from lipid peroxidation and can be estimated to determine the extent of tissue damage (Davey *et al*., 2005). A previous clinical investigation by Verma *et al*. (2019) showed that MDA concentrations were high in hypertensive patients than in normal individuals. This current study corroborated this finding and suggested the extracts inhibited lipid peroxidation further enhancing the antioxidant system. Generation of hydrogen peroxide was also significantly inhibited especially in the kidneys.

In conclusion, the antihypertensive effect of *L. breviflora* whole fruit or *X. sagittifolium* corm extract was demonstrated with effective reduction in blood pressure and was comparable to lisinopril. The extracts also reversed hypoproteinaemia with the consequently increased circulating protein thiols, while enhancing the antioxidant systems of the glutathione enzymes, nitric oxide and catalase. The extracts also ameliorated oxidative damage associated with hypertension determined in this study by decreased MDA levels and hydrogen peroxide activity. Further study is needed to elucidate the bioactive principle(s) responsible for this pharmacological activity.

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Full length Research Article

Mucuna pruriens Seed Repeals the Hypothalamic-Pituitary-Testicular Axis Disruption following Carbamazepine Treatment in Male Wistar Rats

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Summary: This study examined the potential effects of *Mucuna pruriens* (MP) seed powder on the disruptions of the hypothalamic-pituitary-testicular axis caused by the carbamazepine (CBZ) treatment in male Wistar rats. A total of 35 male Wistar rats were randomized into 5 groups (n=7). The animal in group 1 received normal saline (0.2 ml) orally, while groups 2-5 received carbamazepine (CBZ) 25 mg/kg per oral. Groups 1, and 2 were fed with standard rats' chow, while groups 3-5 rats were supplied with a diet containing MP seed powder at 2.25 g, 1.5 g, and 0.75 g respectively. The serum levels of male reproductive hormones [gonadotropic releasing hormone (GnRH), follicle-stimulating hormone (FSH), testosterone, and estradiol] were determined using enzyme-linked immunosorbent assay; seminal profile was evaluated by determining the sperm count, morphology, and viability; the testicular tissue lipid peroxidation was delineated by conventional spectrophotometric assay; while the histomorphology of the hypothalamus, pituitary, and testis was delineated using conventional hematoxylin and eosin staining technique. Descriptive and inferential statistics were used to analyze the result. There was a marked decrease in the testicular weight, FSH, testosterone concentration, and normal sperm cells in the CBZ, and CBZ + MP (2.25 g) treatment groups. There was a marked increase in the testicular tissue lipid peroxidation in the CBZ, and CBZ + MP (2.25 g) treated rats in addition to various morphological alterations in the hypothalamus, pituitary, and testis. These anomalies receded in the CBZ + MP (1.5 g), and CBZ + MP (0.75 g) treatment groups. Consumption of MP (1.5 g, and 0.75 g) may alleviate the injurious effects of CBZ treatment on the hypothalamic-pituitary-testicular functions.

Keywords*: Mucuna pruriens;* Carbamazepine; Hypothalamic-pituitary-testicular axis

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INTRODUCTION

Epilepsy is one of the most serious neurological disorders that affects around 46 million people worldwide (Beghi, 2020). The pathophysiology of seizure is characterized by an occurrence of anomalous neuronal firing due to disequilibrium in the excitation and inhibition mechanisms in the brain (Stafstrom and Carmant, 2015). Carbamazepine, a sodium-gated channel blocker is one of the most popular anticonvulsant drugs; it is often required for long-term treatment of epilepsy because it only recedes the symptoms of epilepsy (seizure), not epilepsy which is a chronic disorder.

Accumulating shreds of evidence have shown that epilepsy is a debilitating disorder while its treatment is with attendant abnormal sexual function (Atif *et al*., 2016; Nikoobakht *et al*., 2017; Mazdeh *et al*., 2020). Both clinical and experimental investigations showed that the hypothalamic-pituitary-testicular axis disruption remains a pivot of reproductive dysfunctions with eventual manifestations of poor sexual performances, loss of libido, and ultimately, infertility (Semet *et al*., 2017; Zhao *et al*., 2019; Osuntokun *et al*., 2020).

Among other indigenous plants consumed as herbal formulations to cure some male reproductive disorders in some developing countries e.g., Nigeria is *Mucuna pruriens* (M.P) (Sofowora 1982); Ashidi *et al*., 2019). It is popularly called velvet bean/ devil bean and also referred to as 'werepe', 'agbada/ Agboloko', and 'karara' among the Yoruba, Igbo, and Hausa tribes of Nigeria respectively. M.P plant belongs to a Fabaceae and it is common in South Western Nigeria (Vadivel and Janardhanan, 2000). Studies on the phytochemical screening of *Mucuna pruriens* seed showed a high concentration of cardiac glycosides, phenols, flavonoids, terpenoids, alkaloids, and coumarins (Ravikumar and Ramachandra, 2020). As a result of the presence of L-3,4-dihydroxyphenylalanine (L-DOPA) in the seed of MP as its active compound, findings from human

and animal models have revealed how *Mucuna pruriens* seed improves reproductive performances (Ahmad *et al*., 2008; Mutwedu *et al*., 2019).

A significant number of men living with epilepsy experience sexual dysfunction (Nikoobakht *et al*., 2007; Zhao *et al*., 2019), while the attendant adverse effects of anticonvulsant drugs stem from erectile dysfunction, reduction in sexual desire to alterations of ejaculation (Yang and Wang, 2016). Additionally, traditional anticonvulsant drugs such as sodium valproate, carbamazepine, phenytoin, etc. have been associated with impairment of the hypothalamic-pituitary-testicular axis (Osuntokun *et al*., 2017; Osuntokun *et al*., 2020). However, the management of epilepsy or its co-morbidity (e.g., reproductive disorder) using orthodox medicine is neither easily assessable nor effortlessly affordable by citizens of many developing countries (Birbeck 2010; Ashidi *et al*., 2019); it is, therefore, imperative to investigate the scientific mechanism of actions of some of the natural plants with acclaimed fertility enhancement on the deranged hypothalamic-pituitary-testicular axis following chronic treatment with an anticonvulsant drug hence, this study.

MATERIALS AND METHODS

Seed collection and preparation: The procurement of the seeds of *Mucuna pruriens* (L.) DC, Var, leaf identification, authenticated, and extraction has been reported in our previous study Osuntokun *et al*. (2021), while Ashidi *et al*. (2019) also reported the phytochemical composition of the seed powder.

Experimental Animal: A total of 35 male Wistar rats procured from the animal house of the College of Health Sciences, Osun State University, Osogbo, Nigeria was randomly assigned into 5 groups (n=7). Group 1 received normal saline (0.2 ml) orally, while groups 2-5 received carbamazepine (CBZ) 25 mg/kg per oral. Groups 1, and 2 were fed with standard rats' chow, while groups 3-5 rats were fed with diets containing MP seed powder at 2.25 g, 1.5 g, and 0.75 g respectively (Ashidi *et al*., 2019). This study was carried out in agreement with the provisions established by the Health Research Ethics Committee (HREC) of the Osun State University, Osogbo, Nigeria in line with the Guidelines for Proper Experimental Animal care (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011).

Seminal analysis: Epididymal sperm were collected from the caudal epididymis immediately after the rats were sacrificed. Sperm progressive motility, morphology, count, and viability were determined using conventional methods as described in our previous study (Osuntokun *et al*., 2020). Briefly, The number of motile spermatozoa was calculated per unit area and expressed as percentage motility. Sperm were counted using a counting chamber and the results were expressed as million sperm/ml suspension. Sperm viability was investigated by ensuring uniform spermatozoa smear on slides with eosin/nigrosine stain, while hundred of sperm cells were counted per slide to obtain the percentage of life/death ratio.

Hormone Assay: The releasing hormone from the hypothalamus (gonadotrophic releasing hormone- GnRH), a stimulatory hormone from the anterior pituitary (follicle-stimulating hormone-FSH), and luteinizing hormone- LH)), the testicular hormone testosterone, and serum concentration of estradiol were determined using the enzyme-linked immunosorbent assay method as described in our previous study (Osuntokun *et al*., 2020b).

Assessment of the markers of lipid peroxidation in the hypothalamus, pituitary, and testicular tissues: The activities of reduced glutathione and concentration of the product of lipid peroxidation, malondialdehyde (MDA) were evaluated in the supernatant of the hypothalamic, pituitary, and testicular tissues spectrophotometrically (Osuntokun *et al*., 2021).

Assessment of the morphological profile of the hypothalamus, pituitary, and testis: The brain, epididymis, and testis were preserved in either neutral buffered formalin or Boin's fluid followed by routine laboratory procedures. After staining with hematoxylin and eosin, sections (5 μ) of the processed hypothalamus, pituitary, and testis were viewed under the Leica DM 750 microscope for any signs of morphological aberrations (Osuntokun *et al*., 2020a).

Statistical analysis: Descriptive and inferential statistics were carried out with the aid of a graph pad prism software, while Student–Newman–Keul's test was employed for the post hoc analysis where appropriate. The level of significance was set at $p<0.05$.

RESULTS

The weight of reproductive and accessory organs: There was a significant ($p = 0.0001$) decrease in the testicular weights of CBZ, and CBZ + MP (2.25 g) treated rats compared with the control (Table 1).

Table 4.1
The weight of reproductive and accessory organs

Treatment Group	Testicular weight (g)	Epididymal weight (g)	Seminal vesicle (g)
Control	1.32 ± 0.04	0.30 ± 0.01	0.82 ± 0.14
CBZ	0.94 ± 0.04$^{\alpha}$	0.28 ± 0.01	0.59 ± 0.08
CBZ + MP (2.25 g)	1.16 ± 0.03 $^{\alpha\beta}$	0.31 ± 0.04	0.47 ± 0.11
CBZ + MP (1.5 g)	1.25 ± 0.08$^{\beta}$	0.29 ± 0.05	0.45 ± 0.04
CBZ + MP (0.75 g)	1.38 ± 0.03$^{\beta}$	0.32 ± 0.01	0.70 ± 0.11

α: decrease compared with the control (p = 0.0001)
β: increase compared with CBZ (p = 0.0006)

The hypothalamic, pituitary, and testicular hormones concentration: The serum concentration of gonadotropic-releasing hormone (GnRH) increased significantly ($p = 0.0180$) in the CBZ + MP (0.75 g) treatment group. There was a significant ($p = 0.0001$) decrease in the serum concentration of follicle-stimulating hormone (FSH) following CBZ treatment, while this hormone significantly ($p = 0.0003$) increased in the CBZ + MP treatment groups relative to CBZ-treated rats. The luteinizing hormone (LH)

concentration increased markedly (p = 0.0055) in the CBZ + MP (0.75 g). The serum concentration decreased significantly (p = 0.0001) in the CBZ treatment. However, testosterone concentration increased significantly (p = 0.0008) across the CBZ + MP treatment groups compared with the CBZ-treated rats (Table 2).

The serum concentration of estradiol: There was a significant increase (p = 0.0003) in the concentration of estradiol in the CBZ treatment groups, while the estradiol concentration decreased significantly (p = 0.0015) compared with CBZ treated rats (Figure 1).

Table 2
The hypothalamic, pituitary, and testicular hormones concentration

Treatment Group	GnRH	FSH	LH	Testosterone
Control	43.90 ± 0.93	0.41 ± 0.01	0.34 ± 0.03	0.36 ± 0.03
CBZ	41.64 ± 5.72	0.29 ± 0.02$^{\alpha}$	0.34 ± 0.01	0.20 ± 0.01$^{\alpha}$
CBZ + MP (2.25 g)	44.60 ± 4.03	0.44 ± 0.03$^{\delta}$	0.38 ± 0.03	0.38 ± 0.02$^{\delta}$
CBZ + MP (1.5 g)	45.03 ± 4.40	0.40 ± 0.04$^{\delta}$	0.36 ± 0.05	0.36 ± 0.04$^{\delta}$
CBZ + MP (0.75 g)	49.91 ± 1.80$^{\beta\delta}$	0.52 ± 0.04$^{\beta\delta}$	0.45 ± 0.02$^{\beta\delta}$	0.43 ± 0.05$^{\delta}$

β: increase compared with control (p = 0.0180)
α: decrease compared with control (p = 0.0001)
δ: increase compared with CBZ (p = 0.0003)

The semen parameters: The percentage of sperm motility declined (p = 0.0001) across the treatment group compared with the control. Also, the percentage of sperm viability decreased markedly (p = 0.0001) across the treatment group except for the CBZ + MP (0.75 g) treatment group. The sperm count of the CBZ treated rats decreased significantly (p = 0.0044). The percentage normal morphology decreased markedly (0.0001) in the CBZ and CBZ + MP (2.25 g) treatment group. (Table 3).

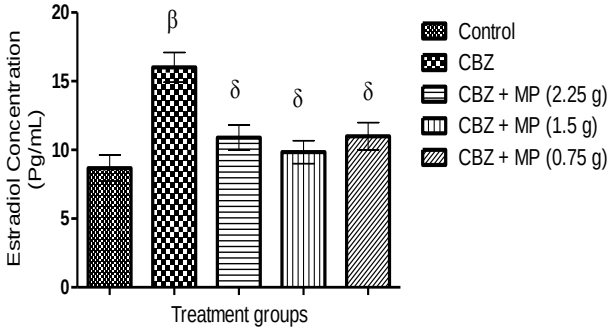

Figure 1
The serum concentration of estradiol
β: increase compared with the control (p = 0.0001)
δ: decrease compared with CBZ (p = 0.0031)

Percentage of sperm morphological alterations: The number of spermatozoa with the observable feature of head defect increased significantly in the CBZ and CBZ + MP (2.25 g) treatment groups. The number of sperm cells with tail defect increased significantly (p = 0.0001) across the treatment group except for CBZ + MP (0.75 g) treated rats. Moreover, the number of sperm with the head only increased significantly (p = 0.0001) in the CBZ, and CBZ + MP (2.25 g) treated a headless body which increased significantly (p = 0.0001) in the CBZ, and CBZ + MP (2.25 g) (Table 4).

The markers of oxidative stress in the testis: This study shows a significant (p = 0.0001) increase in the activities of reduced glutathione in the testicular tissue of all the treatment groups except the CBZ-treated rats. However, the testicular concentration of MDA increased significantly (p = 0.0001) in the CBZ, and CBZ + MP (2.25 g) (Figure 2).

Table 3
The semen parameters

Treatment Group	% Motility	% Viability	Sperm count (10^6 X ml)	% Normal morphology
Control	86.60 ± 2.29	97.00 ± 0.55	112.40 ± 7.74	88.60 ± 1.36
CBZ	19.00 ± 5.57$^{\alpha}$	25.00 ± 2.04$^{\alpha}$	69.80 ± 10.20$^{\alpha}$	18.00 ± 0.71$^{\alpha}$
CBZ + MP (2.25 g)	12.50 ± 1.44$^{\alpha}$	37.00 ± 5.15$^{\alpha}$	93.20 ± 13.95	68.00 ± 6.77$^{\alpha\beta}$
CBZ + MP (1.5 g)	52.50 ± 13.31$^{\alpha\beta\delta}$	61.75 ± 13.80$^{\alpha\beta}$	97.20 ± 6.19	75.60 ± 7.76$^{\beta}$
CBZ + MP (0.75 g)	72.00 ± 3.39$^{\alpha\beta\delta}$	81.25 ± 1.25$^{\beta\delta}$	129.60 ± 8.51$^{\beta}$	78.60 ± 3.01$^{\beta}$

α: decrease compared with control (p = 0.0001)
β: increase compared with CBZ (p = 0.0001)
δ: increase compared with CBZ + MP (2.25 g) (p = 0.0006)

Table 4
Effects of dietary meal supplemented with *Mucuna pruriens* seed powder on the characteristics of carbamazepine-induced sperm morphological alteration in male Wistar rats.

Morphological Parameters	Control	CBZ	CBZ + MP (2.25 g)	CBZ + MP (1.5 g)	CBZ + MP (0.75 g)
Head defect	1.80 ± 0.20	2.60 ± 0.24$^{\beta}$	2.60 ± 0.24$^{\beta}$	1.80 ± 0.20	2.00 ± 0.41
Midpiece defect	2. 80 ± 0.37	3.60 ± 0.51	3.40 ± 0.40	2.60 ± 0.24	2.00 ± 0.41
Tail defect	3.80 ± 0.37	13.60 ± 0.68$^{\beta}$	12.60 ± 1.12$^{\beta}$	13.00 ± 0.71$^{\beta}$	10.20 ± 0.92$^{\beta\mu}$
Head only	1.80 ± 0.37	8.40 ± 0.40$^{\beta}$	6.25 ± 0.95$^{\beta}$	3.40 ± 1.03	1.80 ± 0.66
Headless	1.40 ± 0.51	6.60 ± 0.24$^{\beta}$	7.25 ± 0.95$^{\beta}$	1.60 ± 0.68	1.80 ± 0.49

β: increase compared with control (p = 0.0001)
μ: decrease compared with CBZ (p = 0.0175)

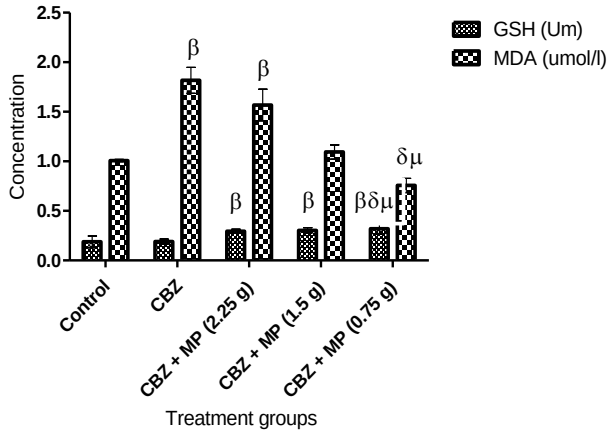

Figure 2
Oxidative stress in the testis
β: increase compared with the control (p = 0.0001)
δ: decrease compared with CBZ (p = 0.0013)
μ: decrease compared with CBZ + MP (2.25 g) (p = 0.0061)

The histomorphology of the hypothalamus
Carbamazepine chronic treatment induced neuronal necrosis (White arrow) which was attenuated following MP (2.25 g) treatment (Blue arrow) (Plate 1).

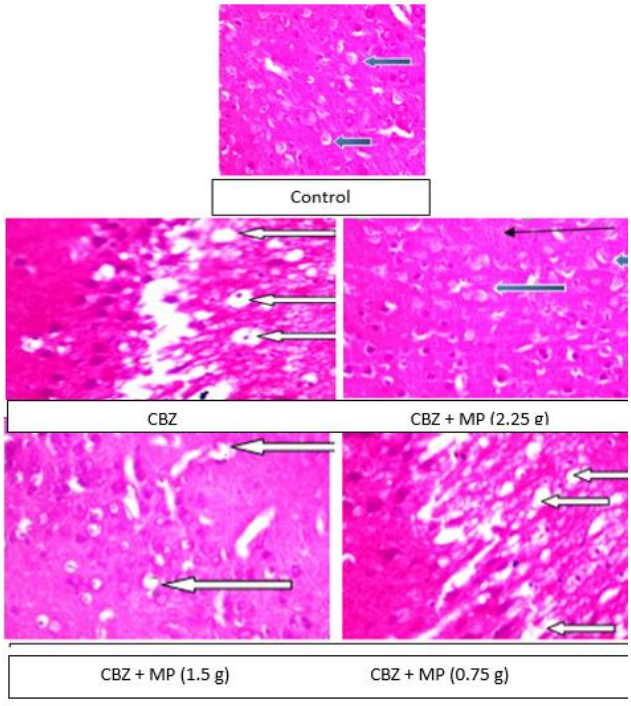

Plate 1
The histomorphology of the hypothalamus
Blue arrow: Normal neuron; Slender arrow: normal stroma; White arrow neuronal necrosis; magnification: 400

The histomorphology of the pituitary
There was marked neuronal vacuolation in the CBZ treated group. However, MP (2.25 g), and MP (1.5 g) alleviated this (blue arrow) (Plate 2).

The histomorphology of the testis
Plate 3 below is the photomicrograph of the testicular section. there was normal histoarchitecture in the testis of the normal saline-treated rats. This was evident with the appearance of the normal seminiferous tubule containing mature spermatozoa within their lumen (white arrow). Several tubules in the CBZ-treated rat are seen with maturation arrest (black arrow), and congested interstitium (slender arrow). The interstitial spaces of the CBZ + MP (2.25 g), and CBZ + MP (1.5 g) showed normal Leydig cells, but with vascular congestion (slender arrow). However, there was no difference in the morphology of the testis of the CBZ + MP (0.75 g) and the normal saline (control) treatment group.

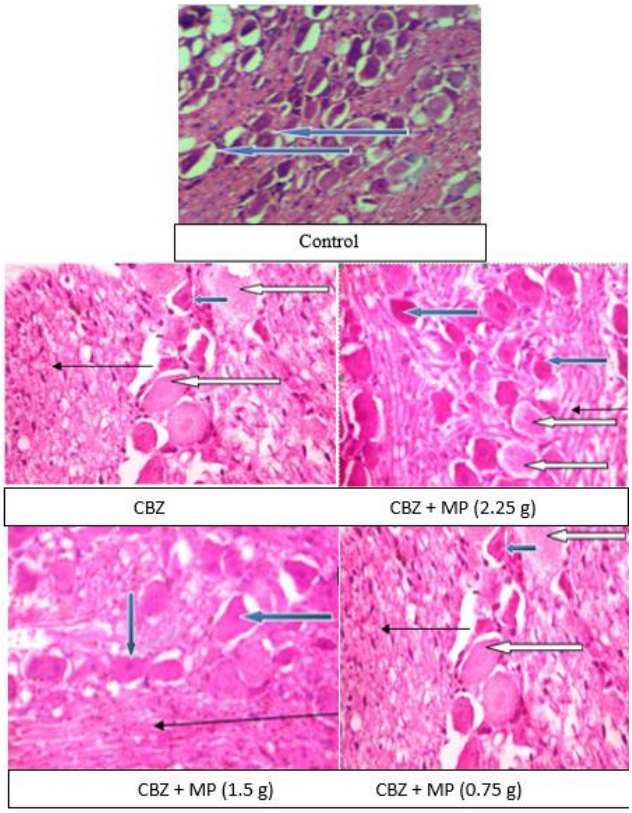

Plate 2
Effects of carbamazepine on the histomorphology of the pituitary gland in male Wistar rats fed dietary meal supplemented with *Mucuna pruriens* seed powder.
White arrow: neuronal: Neuronal vacuolation; Slender arrow: Normal stroma; Blue arrow: normal neuron; magnification: 400

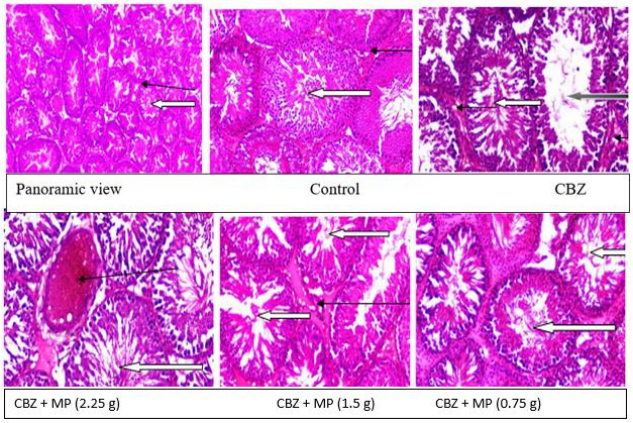

Plate 3
Effects of carbamazepine on the histomorphology of the testis in male Wistar rats fed dietary meal supplemented with *Mucuna pruriens* seed powder.
Magnification: X400; Stain: Haematoxylin and eosin

DISCUSSION

While there are different levels of reproductive toxicity as a response to diverse classes of anticonvulsant drugs, previous studies have shown that the worse sexual function scores were seen in patients or animals treated with sodium valproate, CBZ, phenytoin, etc. compared to some newer anticonvulsant agents like levetiracetam (Mazdeh *et al*., 2020; Osuntokun *et al*., 2020a&b); which are not better off than the former in terms of efficacy (Li *et al*., 2014; Suresh *et al*., 2015). Therefore, since the treatment with some conventional anticonvulsant drugs remains the mainstay in chronic epilepsy, despite their untoward adverse effects, there is a need to investigate the therapeutic effects of some medicinal plants such as *Mucuna pruriens* seed as adjunctive treatment with carbamazepine.

In this present study, neither seminal vesicle nor epididymal weight was affected by the CBZ treatment. However, a significant decrease in testicular weight recorded against CBZ treatment could not be receded completely by the MP (2.25 g) in contrast to what is obtainable in the CBZ + MP (1.5 g), and CBZ + MP (0.75 g) groups. This may serve as potential evidence that the compounds of MP seed at the highest dose (2.25 g) may cause some biological effects which might have interfered with the efficacy of the result. This finding is consistent with our result obtained from the concentration of GnRH analysis, where the lowest dose of MP (0.75 g) tends to be efficacious compared to the other treatment groups.

In this present study, however, although CBZ had no significant effect on the reproductive hypothalamic hormone, an increase in the secretion of GnRH induced by the MP (0.75 g) treatment had a direct and positive influence on the serum concentration of the FSH (despite the adverse effect of CBZ), and LH; while an increase in the serum concentration of testosterone is another suggestive potential evidence that the influence of MP is gonadotropic.

It is a known fact that L-3,4dihydroxyphenylalanine (L-DOPA) is an active substance of *Mucuna pruriens* seed with levels ranging up to 13% (Deli *et al*., 2021). Meanwhile, this substance (L-DOPA) has been implicated as a potent secretagogue of GnRH, with consequential stimulation of the FSH and LH secretion from the anterior portion of the pituitary gland, and eventual testosterone production (Singh *et al*., 2013; Marques *et al*., 2018). This could serve as an evidential (scientific) justification for the aphrodisiac properties of the *Mucuna pruriens* seed as reported by several authors (Ahmad *et al*., 2008; Mutwedu *et al*., 2019). However, despite a high concentration of L-DOPA in the 2.25 g of MP consumed by the CBZ-treated rats, there was no significant difference in the concentration of GnRH between the CBZ and CBZ + MP (2.25 g) treated rats; a suggestive indication that MP (2.25 g) might have posed additional toxicity to the CBZ-treated. Moreover, it is worthy of note that the concentration of L-DOPA in *Mucuna pruriens* at 0.75 g, an equivalent of 1 seed of M.P (Ashidi *et al*., 2019) appears adequate in the induction of GnRH secretion from the hypothalamus and sufficient to attenuate the inhibitory influence of CBZ on the FSH, LH and testosterone secretion

Our finding shows a decrease in the serum concentration of testosterone and an increased level of estradiol in the CBZ-treated rats; this is an assertion of the involvement of CBZ in the induction of enzyme aromatase, an enzyme whose activities convert androgens to estrogens (Jacobsen *et al*., 2008). Additionally, it is evident from this study that MP is a potential therapeutic agent that could attenuate the activities of the enzyme aromatase in the conversion of testosterone to estradiol. This may not be unrelated to a high concentration of alkaloids, tannins anthraquinones, saponins, flavonoids, and cardiac glycosides (Vadivel and Janardhanan, 2000). This finding is consistent with the report of Golan *et al*., (2008) that the phytochemicals, including steroidal saponins and flavonoids Tarigan *et al*., 2016), attenuate estrogen production by inhibiting the action of aromatase.

Our result on the sperm parameters (percentage motility, viability, morphology, and sperm count) reflects the spermicidal effects of CBZ (tables 3 and 4). However, MP supplement in the CBZ + MP (1.5 g) treatment group averted the CBZ-toxicity on the seminal profile, while these adverse effects not only receded in the CBZ + MP (0.75 g) but the indices of normal seminal profile increased significantly compared to the control. While it remains undoubted that the compounds in MP seed increased the testosterone level through hypothalamic-pituitary-testicular axis stimulation; the highest treatment dose in this study (CBZ + MP (2.25 g) posed a deleterious effect, worse than what is obtainable in the CBZ alone treatment group.

An increase in the concentration of malondialdehyde in the testicular tissue of CBZ-treated rats recorded in this present study is a pointer to the potential toxicity posed by CBZ treatment. Despite an increase in the activities of reduced glutathione in the testicular tissue of the CBZ + MP (2.25 g) treatment group, the concentration of malondialdehyde remains very high; suggestive evidence of (1) innate adaptive mechanism of the tissue to oxidative stress posed by the CBZ, and/ (2) the potential toxicity of the MP (2.25 g) which may have been heightened by the CBZ + MP (2.25 mg/kg) combination. However, MP (1.5 g), and MP (0.75 g) doses are more tolerated and therapeutic than the highest dose MP (2.25 g).

The neuronal necrosis and vacuolation observed in the hypothalamus and pituitary of the CBZ treatment group may be partly contributed to a decrease in the FSH, and LH secretions. Therefore, it could be posited that a decrease in the serum concentration of testosterone recorded in the CBZ-treated rat in this study is indirectly caused by damage to the hypothalamus and or the anterior pituitary gland. Apparently, this damage is ameliorated in the CBZ + MP (2.25 g), CBZ + MP (1.5 g), and CBZ + MP (0.75 g) treatment groups in descending order, a factor substantiating the potential toxicity of MP (2.25 g). Moreover, maturation arrest and interstitial space/ vascular congestion, a sign of testicular toxicity following CBZ administration in this present study are consistent with our previous findings (Osuntokun *et al*., 2017; Osuntokun *et al*., 2020). Additionally, Ashidi *et al*. (2019) reported a possibility of alteration in the spermatogenesis of male rats fed with 2.25 g (3 seeds) of M.P seed. This is therefore an assertion that M.P (2.25 g) may not ameliorate the damaging effects posed by CBZ but rather compound it. However, it is worthy of note, that CBZ + MP (1.5 g), and CBZ + MP (0.75 g) treatment groups had relatively normal testicular histoarchitecture, which was devoid of morphological

aberrations in contrast to what was found in the CBZ and CBZ + M.P (2.25 g) treated groups.

In conclusion, this study revealed that the hypothalamic-pituitary-testicular axis derangement induced by carbamazepine toxicity may be attenuated by a moderate or low quantity of *Mucuna pruriens* seed supplement. MP seed enhanced the indices of reproductive function in male Wistar rats by increasing the GnRH, FSH, and LH; and decreasing the serum concentrations of estradiol due to the presence of L-DOPA. The M.P seed also repealed the disruption of the CBZ-induced seminal profile, and histomorphology of the hypothalamus, pituitary, and testis due to its antioxidative mechanism as evident in the activities of the reduced glutathione. However, it is expedient in our future studies to examine the effects of MP on the immunohistochemistry of the hypothalamic-pituitary-testicular axis following CBZ treatment.

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Full length Research Article

Detrimental Effects of *Saccharum officinarum* Juice on Reproductive Functions of Female Wistar Rats

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Summary: Changing dietary compositions have contributed to the growing epidemic of metabolic diseases with serious impacts on several aspects of health, including reproductive health. *Saccharum officinarum* juice has a natural sweetness that makes the general populace relinquish its use as a sweet course and well-known raw material for the production of refined sugar. Studies have reported adverse effects of this juice on male reproductive functions, but there is a paucity of information on females. This study investigated the effects of fresh *Saccharum officinarum* juice on the reproductive functions of female Wistar rats. A sugarcane press juicer was used to extract *Saccharum officinarum* juice. Twenty female Wistar rats (180-200 g) grouped into four (n = 5) received 1.0 mL/kg/day distilled water (control), and 1.0, 3.2, and 10.0 mL/kg/day of fresh *Saccharum officinarum* juice once daily for 21 days by gavage. Serum follicle-stimulating hormone, luteinizing hormone, and estrogen levels were measured using enzyme-linked immunosorbent assay (ELIZA). The estrous cycle was assessed using the Marcondes principle and histology of the ovary and uterus were assessed by microscopy. Data were analyzed using the Analysis of variance at a significance of $p < 0.05$. *Saccharum officinarum* juice caused an increase in the body weight and serum levels of follicle-stimulating hormone and luteinizing hormone. It altered the estrous cycle by increasing the frequency of occurrence of the proestrus phase but reduced that of the metestrus phase. The juice altered the cytoarchitecture of the ovaries via vacuolations and cysts within the ovarian stroma, while the uterine section showed distorted endometrial lining and glands. *Saccharum officinarum* juice inflamed the ovaries and distorted the estrous cycle and uterine endometrial lining. *Saccharum officinarum* juice consumption may possess deleterious effects on the reproductive functions of female Wistar rats.

Keywords*: Saccharum officinarum juice, Ovary, Uterus, Estrous cycle, Infertility.*

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INTRODUCTION

The inability to conceive by natural means after one year of unprotected sexual intercourse is an issue of global concern (Ombelet *et al*., 2008; WHO, 2018), and it is one of the major causes of unhappy marriages (Inhorn *et al*., 2015; Bakhtiyar *et al.,* 2019; Walker and Tobler, 2022). Though male infertility contributes to some cases of global childlessness, female infertility results in profound social consequences for women regardless of the cause of infertility (Inhorn, 2003; Rutstein and Shah, 2004; Bakhtiyar *et al.,* 2019). The human diet has been related to adverse effects on fertility (Panth *et al.,* 2018: Skoracka *et al.,* 2021). Changing dietary composition has contributed to a growing epidemic of metabolic diseases with serious impacts on several aspects of health, including reproductive health (Norman *et al.,* 2004; Salas-Huetos *et al.,* 2017; Silvestris *et al.,* 2019).

Saccharum officinarum (sugar cane) is commonly consumed as a staple food in some parts of the world, and it is a popular raw material for the production of refined sugar **(**Singh, *et al.,* 2015**).** The natural sweetness of the juice makes it attractive for use as a sweet course. Previous studies showed that *Saccharum officinarum* contains important phytochemicals (such as phenolic compounds, sterols, and policosanols) with antioxidant activity, cholesterol-lowering properties, and other potential health benefits (Singh *et al.,* 2015; Adjatin *et al.,* 2019) that are vital in the treatment of several ailments in numerous parts of the world, such as arthritis, boils, colds, cough, dysentery, fever, sore throat, diuresis (Karthikeyan and Simipillai, 2010; Singh *et al.,* 2015; Ali *et al.,* 2019).

On the other hand, *Saccharum officinarum* contains a high amount of sucrose and some studies have reported harmful consequences of high sucrose intake such as ovarian dysfunction in rats (de Melo *et al.,* 2021). Adekunbi *et al.,* (2016) reported that high sucrose and high salt diet adversely altered sperm functions in Sprague-Dawley rats by reducing testicular weight and testosterone hormone levels. The study also revealed that consumption of *Saccharum officinarum* juice in male rats adversely altered reproductive functions by reducing sperm quality and disrupting testicular architecture (Ogunwole *et al.,* 2020).

However, there is a scarcity of evidence correlating the reproductive effects of consuming *Saccharum officinarum* juice in females. This study investigated the effects of fresh *Saccharum officinarum* juice on the reproductive functions of female Wistar rats.

MATERIALS AND METHODS

Preparation of *Saccharum officinarum* juice: The *Saccharum officinarum* plant was obtained from Karu, Nasarawa State, Nigeria. It was authenticated at the herbarium of the Department of Botany, University of Ibadan, and assigned the identification number UIH No.: 22613. The cane of *Saccharum officinarum* was stripped off its leaves and stalk. It was cut into small sizes of 1 cm each and the diced sugar cane was crushed using a sugar cane juicer (Vevor, 110LBS/H, China) to obtain the juice. The juice was filtered with a muslin cloth. The filtrate was subjected to phytochemical screening using a standard procedure (Odebiyi and Sofowora 1978; Sofowora, 1993). The rats were administered the fresh juice daily by oral gavage.

Experimental animals and design: All procedures involving the use of animals were according to the EU Directive 2010/63/EU for animal experiments and the study conformed with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guide-line (2010) and ethical standards of the Department of Physiology, Bingham University for animal experiment. Twenty female Wistar rats (180-200 g) obtained from the National Veterinary and Research Institute, Plateau State were used for this experiment. The rats were kept in Bingham University animal house under standard laboratory conditions and were given feed and water *ad libitum*. The animals were acclimatized for two weeks before the experiment began. They were randomly divided into four groups (n = 5) and received 1.0 mL/kg/day of distilled water (control), 1.0, 3.2, and 10.0 mL/kg/day of SOJ respectively. The dosage regime was in line with the Organization for Economic Co-operation and Development (Ogunwole *et al.*, 2020; OECD, 2022). The administration was done orally once daily for 21 days, body weights were recorded once a week and on the last day of administration using an electronic weighing scale (EK5055, China).

Estrous cycle assessment: This was done pre-treatment and during treatment using the Marcondes principle. As the experiment began, the assessment of the estrous cycle was done for three weeks before treatment. The rats were then administered the fresh juice daily as stated in the design for 21 days, with a concurrent assessment of the estrous cycle between the hours of 7:00-8:00 am every morning as described by (Marcondes *et al.*, 2002). Vaginal-smeared slides were viewed under the microscope and the cell morphology was microscopically assessed using x40 magnification. The result of the estrous cycle study of the pre-treated and treated rats was compared.

Blood collection, serum preparation, and organ harvest: At the end of the third week of administration just before the animal sacrifice, the fasted rats were bled at the tail to get a drop of blood for the determination of the fasting blood glucose level using an automated glucometer (Fine test IGM-00178, UK). Afterward, the rats were sacrificed under thiopental anesthesia (Pereda *et al.*, 2006) and dissected along the linea alba of the anterior abdominal wall to the thoracic cavity to expose the heart and the organs. Blood was obtained through the cardiac puncture into plain serum bottles and allowed to coagulate for at least 45 minutes and then centrifuged at 10,000 rpm for 15 minutes to obtain the supernatant (serum) which was stored at -20 °C. Serum levels of follicle-stimulating hormone, luteinizing hormone, and estradiol were assayed with Enzyme-Linked Immunosorbent Assay (ELISA) kits (Fortress Diagnostics, UK). Furthermore, the ovaries and uteri were harvested, freed from adherent tissues, and weighed immediately with a digital electronic scale (Camry EHA501, China). The ovaries and uteri were fixed in Bouin's fluid for histology.

Histology of the Ovary and Uterus: The ovaries and uteri fixed in Bouin's fluid were processed for microscopy. The tissues were embedded in paraffin and sectioned with a microtome to get the 4–5 μm-thick paraffin sections. The dewaxed sections were stained with hematoxylin and eosin and the slides were viewed under a light microscope at 100× magnification.

Statistical analysis: Data were obtained and evaluated with Graph Pad Prism Statistics software (USA) version 5.0 and presented as mean ± standard error of the mean (mean ± SEM). The level of significance was $p < 0.05$.

RESULTS

Phytochemical screening of *Saccharum officinarum* juice: Phytochemical screening test showed the presence of; cardiac glycoside, terpenoids, saponins, and reducing sugar

Table 1.
Phytochemical screening of *Saccharum officinarum* juice.

TEST	RESULTS
Flavonoids	-
Cardiac glycoside	+ + +
Tannins	-
Anthraquinone	-
Saponins	+
Alkaloids	-
Terpenoids	+
Reducing sugars	+

Key= (+) Present; (-) Absent

Effect of *Saccharum officinarum* juice on fasting blood glucose level.:There were no significant differences in fasting blood glucose levels of all the treated groups as compared to the control.

Effect of *Saccharum officinarum* juice on the percentage change in body weight.: There were no significant changes in the body weight of the rats during pre-treatment. However, significant increases ($p < 0.05$) in body weight were observed in the 1.0 and 10.0 mL/Kg/day *Saccharum officinarum* juice-treated rats respectively relative to the control (Figure 2).

Effect of *Saccharum officinarum* juice on estrous cycle : The result showed that during pre-treatment there were significant increases ($p < 0.05$) in the frequency of occurrence of the proestrus, estrous, and metestrus phases, with a significant decrease ($p < 0.05$) in the frequency of occurrence of the diestrus phase of the estrous cycle as compared to their respective controls. Treatment with 10 mg/Kg/day juice increased the frequency of occurrence of the proestrus phase with a decreased frequency of occurrence of the metestrus phase as compared with their respective controls (Table 3). The number of cycles, as well as the length of the estrous cycle of the treated groups, were not significantly different from that of their respective controls (Table 4).

Effect of *Saccharum officinarum* juice on relative organ weight.
The result shows no significant difference in the weights of the ovary and uterus of the treated groups compared with the control.

Effect of *Saccharum officinarum* juice on serum hormone level: The group treated with 3.2 mL/Kg/day of *Saccharum officinarum* juice showed significant increases ($p < 0.05$) in the serum levels of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels when compared with the respective control (Figure 3). There was no significant difference in the serum estradiol hormone level of the treated rats relative to the control (Figure 4).

Table 2.
Effect of *Saccharum officinarum* Juice on relative organ weight

	Organ weight (g)	
	Ovary	**Uterus**
Control	0.108±0.01	0.064±0.02
1.0 mL/Kg/day	0.114±0.01	0.068±0.02
3.2 mL/Kg/day	0.114±0.03	0.064±0.02
10.0 mL/Kg/day	0.110±0.00	0.080±0.01

Values represent mean ± standard error of mean, n=5.

Effect of *Saccharum officinarum* juice on the Histology of the Ovary and Uterus: The histology of the ovaries of rats in the treated groups showed vacuolations in the granulosa cell, cysts within the ovarian stroma, and corpus luteum. They also appeared with lymphocyte infiltration, degenerated follicles, and distorted stroma as compared with the ovary of the control group which show normal histology. The uterine sections of the treated groups showed distorted endometrial lining and glands (that were less tubular with distorted cuboidal epithelial cells) as compared with the normal uteri of control.

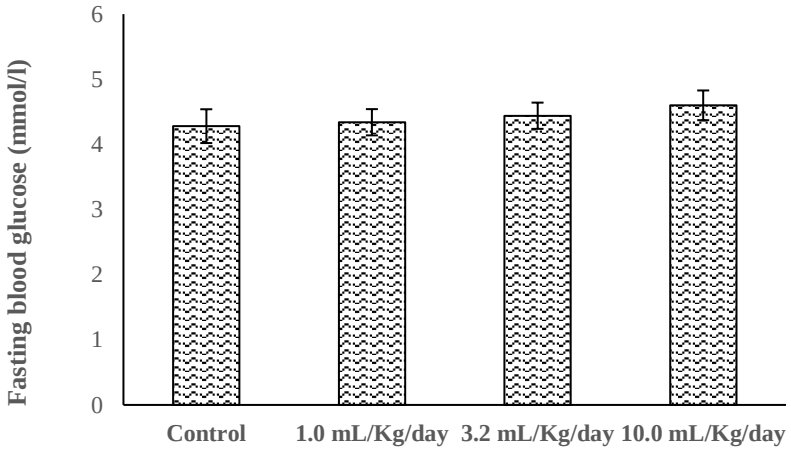

Figure 1.
Effect of *Saccharum officinarum* juice on fasting blood glucose level. Columns represent mean ± standard error of mean, n=5.

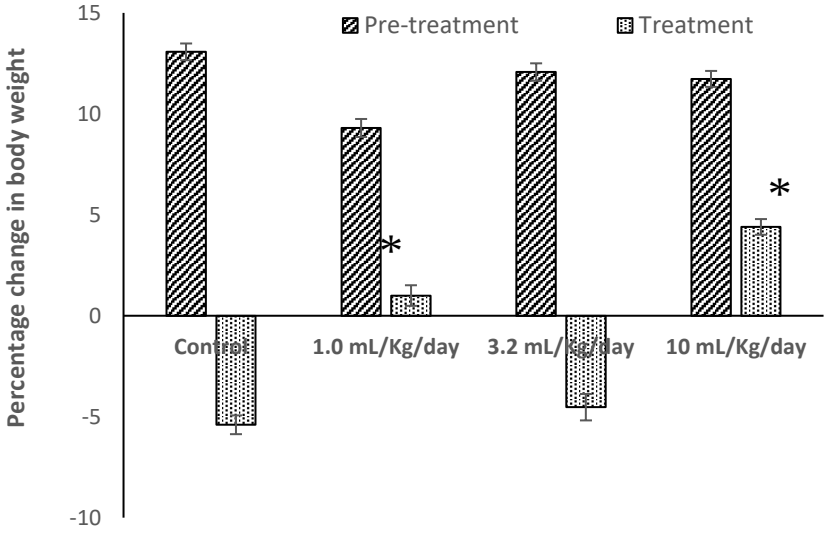

Figure 2.
Effect of *Saccharum officinarum* juice on the percentage change in body weight. Columns represent mean ± standard error of the mean, *$p < 0.05$ compared to control, n=5.

Table 3.
Effect of *Saccharum officinarum* juice on the frequency of occurrence of the phases of the estrous cycle.

	Proestrus		Estrus		Metestrus		Diestrus	
Group	**Pre-treatment**	**Treatment**	**Pre-treatment**	**Treatment**	**Pre-treatment**	**Treatment**	**Pre-treatment**	**Treatment**
Control	20.22 ±4.09	29.22 ±5.65	10.70 ±2.96	13.70 ±2.96	4.49 ±0.23	24.06 ±8.25	65.49 ±4.61	29.62 ±8.56
1.0 mL/Kg/day	20.24 ±2.33	28.58 ±4.00	14.29 ±1.88	6.66 ±1.88	13.1 ±2.32	16.18 ±3.57	52.37 ±1.50	48.58 ±8.85
3.2 mL/Kg/day	28.55 ±5.23	39.00 ±7.53	19.06 ±3.37	10.60 ±3.78	22.63 ±3.5	10.4 ±4.62	29.76 ±4.73	40.0 ±7.09
10.0 mL/Kg/day	41.65 ±5.30*	46.72 ±5.19*	21.45 ±3.19*	15.16 ±3.19	10.41 ±1.03*	12.36 ±5.45*	28.58 ±1.50*	25.76 ±6.25

*Values represent mean ± standard error of mean, n=5, *p < 0.05 compared to control.*

Table 4.
Effect of *Saccharum officinarum* juice on the number and length of the estrous cycle.

Group	Number of cycles		Estrous cycle length (days)	
	Pre-treatment	**Treatment**	**Pre-treatment**	**Treatment**
Control	1.2 ±0.2	1.6 ±0.4	16.2 ±0.2	9.70±0.2
1.0 mL/Kg/day	1.2±0.2	1.0±0.0	10.3±0.3	17.2±0.3
3.2 mL/Kg/day	1.6±0.6	1.4±0.2	11.0±0.3	12.1±0.2
10.0 mL/Kg/day	1.8±0.6	1.4±0.2	5.00±0.3	9.60±0.2

Values Columns represent mean ± standard error of mean, n=5

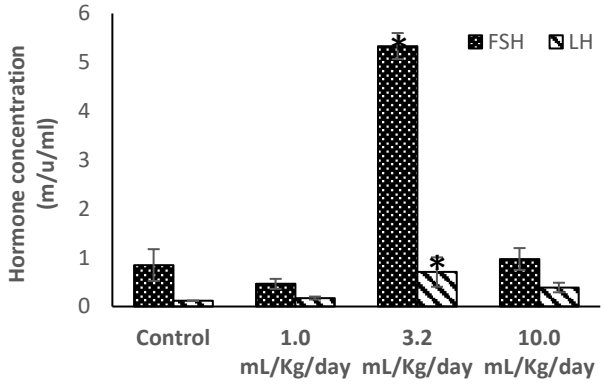

Figure 3.
Effect of *Saccharum officinarum* juice on follicle-stimulating hormone (FSH) and luteinizing hormone (LH) level. Columns represent mean ± standard error of mean, *p < 0.05 compared to control, n=5.

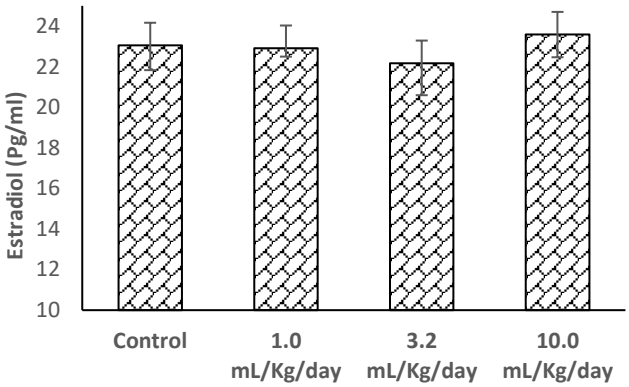

Figure 4.
Effect of *Saccharum officinarum* juice on estradiol level. Columns represent mean ± standard error of mean, n=5.

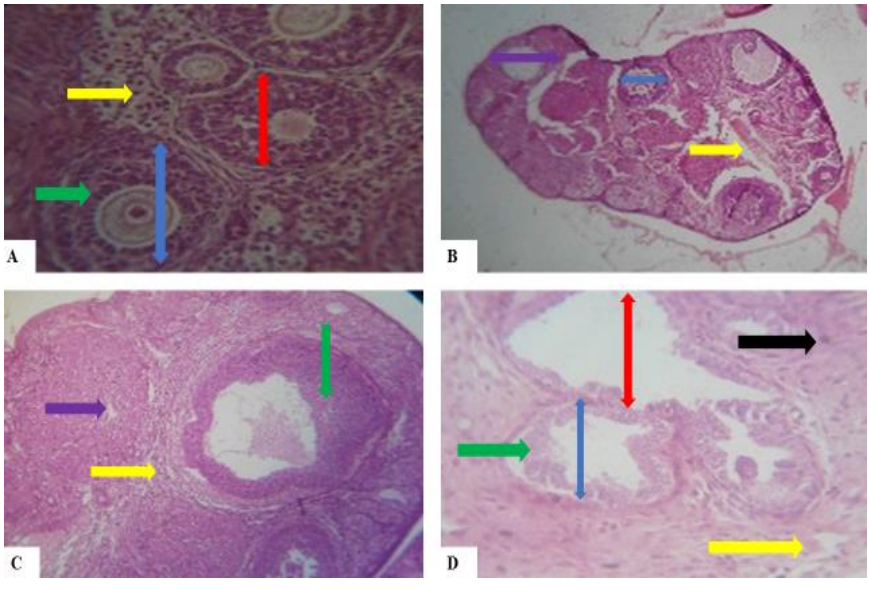

Figure 5a.
Photomicrograph of ovarian sections of control rat and *Saccharum officinarum* juice-treated rats. **A) Control**, appears normal. Note the ovarian primary follicle (red arrow), the secondary follicle (blue arrow), stromal cells (yellow arrow), and well-arranged granulosa cells (green arrow). **B) 1.0 mL/Kg/day of *Saccharum officinarum* juice** Note: vacuolation (purple arrow), cysts within the ovarian stroma (yellow arrow), and degenerated follicles (blue arrow). **C) 3.2 mL/Kg/day *Saccharum officinarum* juice** Note: cysts within the corpus luteum (purple arrow), vacuole in the granulosa cell (green arrow), and distortion of the stroma (yellow arrow). **D) 10 mL/Kg/day *Saccharum officinarum* juice** Note: severe degeneration of the primary follicles (red arrow), secondary follicles (blue arrow), and the granulosa cells (green arrow), lymphocyte infiltration (black arrow), cysts within the secondary follicle (black arrow) and distortion of stroma (yellow arrow). *Stained by H&E. Magnification: x100.*

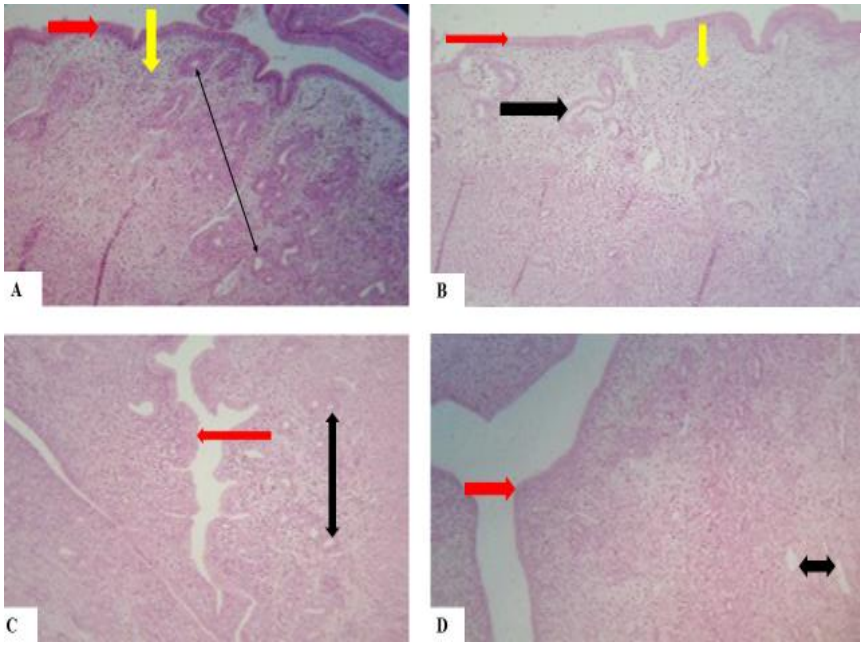

Figure 5b.
Photomicrograph of uterine sections of control rat and *Saccharum officinarum* juice treated rats.
A) Control, appears normal, with endometrium with well-proliferated simple columnar epithelium (red arrow), tubular endometrial glands (black arrow), and lamina propria underlying the endometrial lining (yellow arrow). **B) 1.0 mL/Kg/day of *Saccharum officinarum* juice** Note: endometrial lining (red arrow), distorted endometrial glands (black arrows), and lamina propria underlying the endometrial lining (yellow arrow). **C) 3.2 mL/Kg/day *Saccharum officinarum* juice** Note: a distorted endometrial lining (red arrow), endometrial glands appear less tubular with distorted cuboidal epithelial cells (black arrow). **D) 10.0 mL/Kg/day *Saccharum officinarum* juice** Note: few intact endometrial glands with a distorted base membrane**.**

DISCUSSION

The phytochemicals present in *Saccharum officinarum* juice include cardiac glycoside, terpenoids, saponins, and reducing sugar in line with previous findings that it contains phenolic compounds with great biological activities (De-Armas *et al.*, 1999; Ashade *et al.*, 2014; Ogunwole *et al.*, 2020). The observed inviolate fasting blood glucose level of the treated rats implied that the glycemic index of *Saccharum officinarum* juice was probably adequate to maintain a normal fasting blood glucose level of the female rats during the short period of 21 days juice administration in contrast with Ogunwole *et al.* (2020) who reported increases in fasting blood glucose levels after eight weeks of administration of *Saccharum officinarum* juice to male Wistar rats.

Another factor could be the gender of the rats since hormones in females particularly estrogen has been reported to affect glucose homeostasis (Kumar *et al.*, 2011; Mauvais-Jarvis, 2018). This study showed an increased percentage change in body weight in the *Saccharum officinarum* juice-treated rats, which may be a result of energy gained from the nutrients in the juice and the feed given, supporting Swinburn *et al.* (2004) who noted that weight is gained generally from food consumption. On the other hand, the study disagrees with the findings of Flavel *et al.* (2021) who revealed that polyphenol-rich sugar cane extract reduced body weight in mice fed with high fat, and high carbohydrate diet, and Ogunwole *et al.* (2020) who noted reduced body weights after consumption of *Saccharum officinarum* juice for a longer period in male Wistar rats.

The estrous cycle of rats consists of four phases, usually identified according to cell types observed in vaginal smears. These phases are the Diestrus (resting stage - last for 57 hours), Proestrus (preovulatory stage - last for 12 hours), Estrus (ovulatory stage - last for 12 hours), and Metestrus (degenerative stage - last for 21 hours), (Hebel and Stromberg, 1986). The result showed an increased frequency of occurrence of the proestrus phase and reduced occurrence of the metestrus phase implying that *Saccharum officinarum* juice had an impact on the estrous cycle of the rats. During a normal proestrus phase in rats, there is an increase in follicle-stimulating hormone and luteinizing hormone levels that cause the ovarian follicles to grow faster and a corresponding increase in estradiol level (Hebel and Stromberg, 1986; Maeda *et al.*, 2000). However, in this present study, though the juice administered caused the proestrus phase to appear more frequently and increased the follicle-stimulating hormone and luteinizing hormone levels, it did not alter the estradiol level, indicating an abnormal proestrus phase that was probably either longer or shorter in period and did not lead to the estrus phase. This may be the reason for the metestrus phase that also occurred less frequently as well, resulting in a disrupted estrous cycle. The histology showed alterations in the cytoarchitecture of the ovary and uterus. The observed ovarian lymphocyte infiltration may be from inflammatory reactions that probably occurred in response to the pre-ovulatory gonadotropin stage, preceding ovulation (Oliver *et al.*, 2010). The follicular cells appeared degenerated with distorted granulosa cells and may have resulted from granulosa cell apoptosis due to the presence of Fas antigen, a cell surface receptor protein that is expressed on granulosa cells that mediate signals which induces apoptosis, a vital role in follicular atresia (Sakamaki *et al.*, 1997). The reported vacuolations within the corpus luteum and stroma may be a result of pregnancy failure (Oliver *et al.*, 2023). The noted vacuolations in the endometrial lining and distorted endometrial glands may be caused by atrophy of the uterus. Atrophy is a common age-related change that can be induced by agents that cause ovarian damage. It affects the endometrium and myometrium resulting in fewer endometrial glands and the collapse of the stroma (Davis *et al.*, 1999).

In conclusion, *Saccharum officinarum* juice inflamed the ovaries, distorted the estrous cycle, and caused vacuolations of the uterine tissues. *Saccharum officinarum* juice consumption may possess deleterious effects on the reproductive functions of female Wistar rats.

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ABSTRACTS OF THE PROCEEDINGS OF THE XL[th] ANNUAL SCIENTIFIC CONFERENCE OF THE PHYSIOLOGICAL SOCIETY OF NIGERIA

7-11 February 2023, University of Ibadan, Ibadan, Oyo State, Nigeria.

THE EFFECT OF HIGH PLANT AND ANIMAL PROTEIN DIET ON REPRODUCTIVE INDICES AND TESTICULAR EXPRESSION OF COX-2 AND RHOX5 GENE IN MALE WISTAR RATS.

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High protein diet (HPD) intakes have been reported to be an essential dietary tool in weight loss practices, obesity, dyslipidemia, glucose intolerance, and hypertension. This study was aimed at investigating the possible reproductive effects of high concentrates of plant and animal protein diets in male Wistar rats. Rats were divided into three groups; Control group (CG; fed with normal rat chow), Animal Protein Diet (APD) Group and Plant Protein Diet (PPD) Group. Protein diet groups were further subdivided into three groups (1= 30%, 2 = 50% and 3 = 65% protein concentrates) thatwere fed for duration of one, two and three months respectively (n = 5). At the end of the feeding period, blood samples were collected via cardiac puncture. Epididymis was slit and sperm cells were extracted, testes were harvested for histological and gene studies. ELISA method was adopted for hormonal assay (FSH, LH, and testosterone), sperm count was done using microscope and the sperm cells were counted using haemocytometer. Testicular gene expression of COX-2 and Rhox5 was done using RT-PCR. Both PPD and APD significantly increased sperm count and testicular expression of homeobox5 (Rhox5) and cyclooxygenase 2 (COX-2) genes when compared with control groups ($P<0.05$, respectively). Chronic feeding (3 months duration) with 30% and 50% APD significantly increases testosterone levels and increase in the interstitial cells of leydig. Furthermore, positive correlation exist between COX-2 gene expression and testosterone levels in rats fed with 30% and 50% Protein diets. Conclusively, HPD improves sperm count and increases testicular levels of genes that are implicated in reproductive function.

Keywords: *High protein diet, gene expression, testosterone, homeobox5, cyclooxygenase 2*

KOLAVIRON REVERSES EPITHELIAL INJURY CAUSED BY HEXAVALENT CHROMIUM INGESTION IN THE GUT OF *Drosophila melanogaster*

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The gastrointestinal tract modulates food intake and balance energy distribution throughout the body. In humans, the Drosophila's gut functions may be altered by foreign substances like bacteria and heavy metals. Kolaviron, a bioflavonoid fraction from Garcinia kola seed, has been shown to be gastroprotective and exhibit chelating activities on heavy metals. In this study, we examined some morphological changes in the gut of Drosophila melanogaster during and after exposure to diets-containing chromium (VI) and kolaviron. D. melanogaster (Oregon strain of 1-3 days old of both sexes) were exposed to chromium (VI) (0, 0.05, 0.25, 0.5, 1, 2, 10, 20, 30, 50 and 100 mg/kg diet) and kolaviron (0, 25, 50 and 200 mg/kg diet) for survival assays in two separate studies. Consequently, 50 and 100 mg/kg Kolaviron were selected to evaluate its protective role in chromium (VI) (1 and 2 mg/kg diet)-induced toxicity in D. melanogaster after seven days of oral exposure. Markers of oxidant-antioxidant status (Carbonyl, Nitric oxide, Catalase, Total and Non-protein thiols) were determined by spectrophotometry. Flies' guts were dissected using all-but-gut removal techniques. Histology of the flies' gut using bromophenol blue and H&E stain as well as immunohistochemistry quantification using MitoTraker antibody were evaluated. Data were analyzed using ANOVA and significant at α 0.05. Kolaviron significantly increased flies' survival rate by 87.14%, restored catalase activity and levels of total and non-protein thiols, reduced carbonyl and nitric oxide levels aggrevated by chromium exposure. Altered gut epithelial architecture induced by chromium (VI) exposure were ameliorated by kolaviron. Kolaviron protected against acute and chronic exposure to chromium (VI) by attenuating oxidative markers, and restoring the integrity of the gut.

Keywords: *Drosophila melanogaster, Chromium (VI), kolaviron, Drosophila melanogaster, antioxidants.*

EFFECT OF HIGH DOSE ASCORBIC ACID ON RATE PRESSURE PRODUCT RESPONSE TO AEROBIC EXERCISE IN YOUNG ADULTS

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Rate pressure product is used to indirectly determine the myocardial oxygen consumption and thus cardiovascular risk of subjects. Ascorbic acid is a powerful aqueous-phase antioxidant which scavenges free radicals and reactive oxygen species (ROS) produced during metabolic pathways. Forty-four participants were recruited after giving informed consent. They were grouped into two equal groups of Lean (BMI =21.26±0.48 kg/m^2) and Obese (BMI =33.72±0.76 kg/m^2) participants. Blood pressure (BP), heart rate (HR) and plasma electrolytes were measured before and

after participants performed aerobic exercise using a motorised treadmill for 3-5 minutes. All tests were repeated before and after participants ingested 2000mg of ascorbic acid daily for 3 days. Rate pressure product (RPP) was calculated as systolic (SBP) x HR. Data were expressed as mean±SEM. Unpaired Student t-test and two-way analysis of variance were used to compare across groups; statistical significance was accepted at P<0.05. Baseline SBP, DBP and MABP in LP were significantly lower than those of OP (P<0.0001, P=.003 and P=0.03 respectively). Ascorbic acid led to reduction in SBP (LP to 79±2 mmHg and OP to 116±3 mmHg; p<0.0001); DBP (LP to 71±3 mmHg and OP to 78±2 mmHg; (P=0.003) and MABP (LP to 86±3 mmHg and OP to 92±2 mmHg; p= 0.08). The HR of LP (77±3 b/min) was not significantly less (p=0.11) than that of OP (85±2 b/min). Ascorbic acid reduced HR of LP slightly to 76±3 b/min and that of OP to 81±2 b/min (p=0.12). The RPP of LP was 9036±522 mmHg*b/min was similar to that of OP (10056±326 mmHg*b/min) (p=0.11). Ascorbic acid led to a reduction in the RPP of OP by -7.09±4.14% and in LP by -6.40±5.28% (p=0.92). High dose ascorbic acid had a more pronounced effect on rate pressure product in obese participants than lean participants.

MODULATORY EFFECTS OF CONCURRENT TREATMENT OF CANNABIDIOL (CBD) ON GLUCOSE AND LIPID METABOLISM IN RITONAVIR AND HIGH FAT DIET TREATED ADULT MALE ALBINO WISTAR RATS

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HIV-related lipodystrophies are the most common form of lipodystrophy affecting up to 50 % of HIV patients, owing to the use of protease inhibitors in HIV treatment. Cannabidiol (CBD) has been reported to positively affect metabolic and nervous functions; it is however not clear whether CBD can ameliorate the lipodystrophic effects induced by protease inhibitor (PI). The present study was thus designed to evaluate the modulatory effects of 2 weeks concurrent treatment of CBD on lipid and glucose metabolism in protease inhibited adult male albino Wistar rats. Adult male wistar rats weighing 180-200 g were divided into five groups (n=7). Group 1 animals served as control and were untreated. Group 2-5 were treated with protease inhibitor and high fat diet (HFD) for 2 weeks. Concurrent treatment of apple cider vinegar (0.8 mg/Kg), CBD (10 and 25 mg/kg) were administered to groups 3-5 animals respectively. Glucose and lipid metabolic parameters (such as OGTT, ITT, lipid profile and fasting serum insulin levels), hepatic oxidative stress markers, liver function test and hepatic leptin/JAK 2 expressions were determined. 2 weeks treatment of rats with ritonavir and HFD caused lipodystrophy like symptoms evidenced by hepatic steatosis, hypercholesteremia, impaired liver function, high glucose intolerance and reduced insulin sensitivity. Concurrent treatment of animals with CBD mitigated the ritonavir and HFD induced alteration in glucose and cholesterol metabolism which was associated with improved insulin response and increase in hepatic leptin expression. The beneficial effects of CBD treatment on lipodystrophy appears to involve the suppression in the c-Jun N-terminal kinase (JNK) pathway as animals treated with CBD in the present study had lowered lipid peroxidation and decreased JAK-2 hepatic expression.

Keywords:
Lipodystrophy, Metabolism, Cannabidiol, JAK-2 pathway

BETA- 3 ADRENERGIC DRUGS MODULATE GASTRIC PUMP AND MAST CELL ACTIVITIES DURING HEALING OF ACETIC ACID INDUCED ULCER IN RATS

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The gastrointestinal system functions optimally during homeostatic balance between aggressive and protective factors; disequilibrium in favor of aggressive factors causes gastric ulcer formation. Gastric ulcer remains a significant public health concern worldwide. Mast cells have been investigated to protect the integrity of the gastric epithelium during the inflammatory phase of gastric ulcer repair. Various studies have established the gastro-protective effect of beta-adrenoreceptors, but their role in gastric ulcer healing processes is poorly understood, hence this study. Chronic gastric ulcers were induced using 0.06mls, 60% vol/vol acetic acid, and thereafter treated with Nebivolol 5mg/kg and Carvedilol 12.5mg/kg. Area of the ulcers was measured using planimetry 3 and 7 days later. Gastric Na^+K^+ATPase, H^+K^+ATPase, and Ca^{2+}ATPase pump activities were assessed spectrophotometrically. Histological evaluation and quantification of gastric mast cells on days 3 and 7 of sacrifice. Data obtained were analyzed using ANOVA, significant at α 0.05. There was a significant increase in the level of glutathione concentration and population of the degranulated mast cells in Nebivolol and Carvedilol treated groups when compared with the ulcer untreated group on both days of sacrifice. There was a significant decrease in the gastric H^+- K^+ATPase, while there was a significant increase in the activities of Na^+-K^+ATPase on day 3 and Ca^{2+}ATPase across the treatment groups on both days of sacrifice when compared with the ulcer untreated group. Beta 3 adrenoreceptor agonists improved gastric antioxidant status, augments gastric Na^+-K^+ATPase and Ca^{2+}ATPase pump activities as against H^+-K^+ATPase activity to expedite healing of experimental chronic gastric ulcer.

MODULATORY EFFECTS OF CANNABIDIOL (CBD) ON NF-KappaB p65/RELA SIGNALING PATHWAY IN METABOLIC SYNDROME IN ADULT MALE ALBINO WISTAR RATS

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Earlier studies suggest that Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) Pathway plays

a crucial role in metabolic syndrome due to widespread inflammation in this condition. Cannabidiol (CBD) supplements are Cannabis sativa-derived products with low tetrahydrocannabinol levels, making it non-psychoactive. It has been reported for its anti-inflammatory, anti-oxidant and hypoglycemic effects. However, it is not known whether CBD is capable of inhibiting NF-KB associated inflammatory conditions. The present study was thus designed to investigate the effect of CBD supplementation on olanzapine induced metabolic syndrome in adult male albino Wistar rats. Thirty five (35) adult male Wistar rats were divided into five (5) groups (n=7). Group 1 animals served as control and were untreated. Group 2-5 were induced with metabolic syndrome features by 2 weeks administration of high fat diet (HFD) and Olanzapine (5mg/kg). Concurrent treatment of metformin (20 mg/Kg), CBD (10 and 25 mg/kg) were administered to groups 3-5 animals respectively. Metabolic parameters (such as OGTT, ITT, lipid profile and fasting serum insulin levels), oxidative stress markers, liver function test and hepatic RelA expressions were determined. Two weeks concurrent treatment of olanzapine and HFD induced metabolic syndrome features which were evidenced by hyperglycemia, insulin intolerance, hyperlipidemia, increased serum insulin levels and hepatic oxidative stress. CBD at both doses ameliorated the olanzapine and HFD induced metabolic syndrome features which were associated with significant reduction in hepatic RelA expressions. CBD possesses remarkable metabolic preserving activities which are associated with down regulation of NF-KB activity in rats treated with OLAN+HFD.

Keywords:
Metabolic Syndrome, Olanzapine, CBD, NF-KB

METHANOLIC EXTRACT OF *Ricinus communis* (CASTOR) LEAF AMELIORATED DICHLORVOS-INDUCED CARDIOTOXICITY IN MALE WISTAR RATS

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Accidental poisoning caused by indiscriminate use of organophosphates (OP) has become endemic in recent decades. Evidences from previous studies have revealed that OP poisoning caused cardiovascular abnormalities. However, getting satisfactory antidotes has been a challenge. This study thus evaluated the effect of methanolic extract of *Ricinus communis* on cardiac markers in dichlorvos-exposed Wistar rats. Thirty-two male Wistar rats (200-250g) were randomly assigned into four groups (n=8). Group 1 (control) received 8mL/Kg of Dimethyl sulfoxide dissolved in distilled water. Group 2 were exposed to 1ml of DDVP via inhalation (15mins daily for 4 weeks). Group 3 were exposed to DDVP and then treated with 300mg/kg Castor leaf extract orally for 6weeks. Group 4 received RC only (300mg/kg) orally for 6 weeks. After 42 days, blood pressure and heart rate of the animals were measured in-vitro using tail cuff method. The animals were euthanized; blood was collected via cardiac puncture and centrifuged to obtain plasma for lipid profile, cardiac injury markers and inflammatory markers. Heart tissue was also excised for histology, immunohistochemistry, oxidative stress markers and DNA fragmentation test. Dichlorvo**s** significantly increased (P<0.05) blood pressure, heart rate, inflammatory markers, MDA and DNA fragmentation. However, *RC* significantly reduced (P<0.05) the elevated levels of heart rate, blood pressure, cardiac injury markers, inflammatory markers, MDA and DNA fragmentation. Results from this study suggest that *Ricinus communis* extract exhibits cardio-protective potential.

EFFECTS OF VITAMIN E AND SELENIUM-YEAST ON COGNITIVE PERFORMANCE OF WISTAR RATS SUBJECTED TO PRENATAL NOISE STRESS

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Noise is a common source of environmental stress globally following increased industrialization. It has been shown to induce oxidative stress (OS). Hence there is need for antioxidants supplementation, particularly vitamin E and selenium-yeast to mitigate OS. Twenty-five pregnant Wistar rats were divided into five groups, group 1 (negative control; received 1mL/kg of distilled water without noise stress), group 2: (positive control), received 1mL/kg of distilled water +100 dB/4 h (0900–1300 h) /day of white noise (WN), group 3: vitamin E; 100 mg/kg/day + WN, group 4: Selenium-yeast (0.4 mg/kg/day) + WN and group 5: vitamin E (100 mg/kg/day bw) and selenium-yeast (0.4 mg/kg/day) + WN; all administrations were done 30 minutes before induction of the stress between (0900–1300 h) for 15 days. On gestational day 21. The pups were subjected to cognitive tests using the Y-maze apparatus on days 21 and 22. Tissues were collected for biochemical and immunohistochemical studies. Data were analyzed using one-way ANOVA and Tukey's posthoc test and expressed as mean±SEM with values of p<0.05 as statistical significant. Graphpad prism 8.0. Serum corticosterone (CORT) levels statistically increased in group 2 (p<0.001) compared with group 1, 3, 4, and 5. Group 2 showed an increased level of Malondialdehyde (MDA), decreased levels of glutathione reductase (Gpx), catalase (CAT), and superoxide dismutase (SOD) of brain tissue homogenate compared to group 1. However, most of these changes were mitigated in the antioxidants treated groups. Group 2 shows a moderate proportion of astrocytes cell activation when compared to group 1; this effect was also partly reverted in the antioxidant-treated groups. Prenatal noise stress increases serum CORT, which is associated with increase OS and may

be linked with astrocytes cells activation. However, groups that received vitamin E, selenium-yeast, and combine administration of selenium-yeast and vitamin E showed improvement in cognitive performance.

Keyword: Prenatal stress, Noise, Vitamin E, Selenium-yeast, Astrocytes

THE INFLUENCE OF SMOKELESS TOBACCO CONSUMPTION ON BODY WEIGHT IN RATS

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Tobacco smoking reduces body weight (Audrain-McGovern and Benowitz, 2011). However, the effects of smokeless tobacco consumption on body weight and the factors that may affect body weight have not been fully ascertained. This study therefore examines the effect of consumption of smokeless tobacco on body weight and the factors that can affect body weight namely: food intake, water intake and basal metabolic rate. Twelve male wistar rats (180 – 220g) were randomly assigned to control and test (smokeless tobacco or TCD) groups (n=6). The control group rats were provided with normal rat diet and water *ad libitum* while rats in the test were fed 15% formulated tobacco diet and allowed water for two weeks. The animals were placed in metabolic cages. The animals' body weight, water intake and food intake were measured daily for two weeks. Their basal metabolic rate was also determined by the indirect calorimetric method which measures the amount of oxygen consumption. The results showed that there was a significant decrease in the mean daily food intake in the TCD group (4.26 ± 0.19g) compared to control group (12.19 ± 0.16g) (P< 0.001). Mean daily water intake decreased significantly in the TCD group (6.68 ± 0.28ml) compared to control group (12.93 ± 0.28ml). Mean body weight decreased significantly in the TCD group (181.8 ± 3.29g) compared to control (193.6 ± 2.91g) (P<0.001). There was also a significant reduction in body weight change in the TCD group (-30.00 ± 4.47g) compared to control group (23.33 ± 3.33g) (P<0.001). Basal metabolic rate increased in the TCD group (0.90 ± 0.01 ml/hr/g) compared to control (0.81 ± 0.02 ml/hr/g) (P< 0.001). In conclusion, consumption of smokeless tobacco diet reduces body weight in rats. The reduction in body weight may be attributed to a reduction of water intake, food intake and increase in basal metabolic rate.

AMELIORATIVE ACTIVITIES OF COBALT CHLORIDE ON EXPERIMENTAL CROHN'S COLITIS: ROLE OF MAST CELLS, CA^{2+}ATPASE AND $NA^{+}K^{+}$-ATPASE PUMP ACTIVITY.

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Mast cells have been documented to play valuable roles during healing majorly by mediating wound contraction during healing. Crohn's colitis is a diseased condition of the colon characterized by untamed inflammatory activities. Cobalt chloride has been documented to be gastroprotective and recently enhance gastric motility via increased mechanosensor activities. The activities of Cobalt chloride on colon mast cells during healing of crohn's colitis is vague which this study investigates. 50 male Wistar rats (120 - 130g, n = 10) were divided into 7 groups viz: Groups 1-Control group, 2-Crohn's Colitis Untreated, 3-Crohn's Colitis treated with High Cobalt (62mg/kg), 4-Crohn's Colitis treated with Low Cobalt (25mg/kg), 5-Crohn's Colitis treated with Sulfasalazine (500mg/kg). Crohn's Colitis was induced intra-rectally with NaOH. Daily body weights, colitis score, and colon biochemical analyses were evaluated on days 3 and 7 post-induction of colitis. Histological evaluation of the colon tissue and mast cell counts were quantified. Immunohistochemistry expressions of colon serotonin levels were quantified. Data were expressed as Mean ± SEM and were analyzed using one way ANOVA, $p \leq 0.05$ was considered statistically significant. Body weights significantly increased in the cobalt treated groups. Cobalt treated groups significantly reduced ulcer area and diarrhea score by day 7 compared with colitis untreated group. Cobalt treatment significantly increased levels of colon mucin and Na^{+}/K^{+}ATPase activities but decreased colonic myeloperoxidase level compared with colitis untreated on both days. Colon nitric oxide levels, Ca^{2+}ATPase and mast cells were significantly increased by day 3 but decreased by day 7 on both days during colitis healing in cobalt treated groups compared with colitis untreated. Colon serotonin levels was upregulated in the cobalt chloride treated groups. Cobalt chloride stimulated the healing of crohn's disease in sodium induced experimental rats via increased antioxidant enzymes and modulated mast cell degranulation.

***Key words**: Colitis, Cobalt chloride, mast cell, serotonin expression.*

PRE-TREATMENT OF WISTAR RATS WITH LOW-DOSE CO-ADMINISTRATION OF VITAMIN E AND LITHIUM CHLORIDE REDUCES THE CO-MORBIDITY OF NEUROPATHIC PAIN

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Pain is the most prominent symptom experienced in peripheral neuropathy and lithium chloride (LiCl) has been implicated in treatment of allodynia, hyperalgesia and offers neuroprotection to nerve cells. Vitamin E (Vit E), an antioxidant has been used to reduce hyperalgesia in chronic construction injury. The mechanism by which Vit E, LiCl and their co-administration reduces co-morbidity in neuropathic pain in animal models has not been well studied. A total of 36 male Wistar rats 180-200g of body weight were randomly assigned to seven experimental groups (n=7): Group 1 (Control) was given normal saline, Group 2 (Sham Operated) was also given normal saline, Group 3 (Ligated but untreated) was also given normal

saline, Group 4 (NP) was treated with LiCl 15mg/kg+ Vit E 50mg/kg, Group 5 (NP) treated with 7.5mg/kg LiCl + 50mg/kg Vit E, Group 6 (NP) 4mg/kg LiCl+ 25mg/kg Vit E and Group 7 (NP)7.5mg/kg LiCl+ 100mg/kg Vit E. Neuropathic pain was induced through sciatic nerve ligation in the left leg, while Vit E, LiCl were co-administered for seven days before ligation and administration was continued for 21 days after establishment of Neuropathic pain. All administration was done orally. On the last day of the experiment, the animals were sacrificed. The pre-frontal cortex and spinal cord were collected, homogenized and centrifuged to collect the needed substrates. During the period of treatment, pain behavioral tests (Mechanical allodynia test (MAT), Open field test (OFT), Thermal hyperalgesia test (THT)) were conducted at designated days (Baseline, 3rd day, 7th day, 14th day and 21st day after ligation). To determine the effect of co-administration of Vit E and LiCl on co-morbidity of neuropathic pain, Calcium levels, Dopamine levels and Total protein (TP) levels were assayed. The result of the biochemical analyses showed a significant ($p< 0.05$) increase in dopamine levels in groups treated with 7.5mg/kg LiCl + 50mg/kg Vit E, 4mg/kg LiCl + 25mg/kg Vit E, 7.5mg/kg LiCl + 100mg/kg Vit E compared with the ligated control group. There was significant increase in TP levels across all groups compared with the ligated control group. There was significant increase in calcium levels in group treated with 15mg/kg LiCl + 50mg/kg Vit E compared with the ligated control group. In conclusion, this study demonstrated that in different doses, low dose mixture of LiCl and Vit E reduces pain perception and threshold, thereby reducing the co-morbidities associated with neuropathic pain.

COMPARATIVE ANALYSIS OF THE EFFECT OF AQUEOUS EXTRACT PHYLLANTHUS AMARUS, L-ARGININE AND CHLORAMPHENICOL ON OSMOTIC FRAGILITY ON RED BLOOD CELLS OF DIFFERENT GENOTYPES (HBAA, HBAS AND HBSS)

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Red blood cells of different haemoglobin genotypes (HbAA, HbAS and HbSS) was incubated in *Phyllanthus amarus*, chloramphenicol and L-arginine after which the red cells were subjected to osmotic stress test in phosphate buffered saline. The aim of the experiment was to ascertain/compare the effect of Phyllanthus amarus aqueous extract, L-arginine and chloramphenicol on the integrity of red cell membranes of the different genotypes. Doses of 10 mg and 20 mg/ml of aqueous extract of Phyllanthus amarus, Chloramphenicol and L-Arginine was used for the study. Washed red cells were incubated in each of the drugs/extract for 3 hours before subjecting to osmotic stress in serial dilutions of PBS, for 30 minutes, spun in a bucket centrifuge at 2500 rpm for 10 minutes. Absorbance of the supernatant was measured using a spectrophotometer at 540nm wavelength. Results of percentage haemolysis was analysed against serial dilutions of PBS. Result showed a shift to the left in haemoglobin AA genotype cells incubated in 10mg and 20mg Phyllanthus Amarus, 10mg and 20mg chloramphenicol, 20mg L-Arginine but a shift to the right in cells incubated in 10mg L-Arginine. Same was recorded for Haemoglobin AS genotype red blood cells except for 10mg and 20mg L-Arginine which showed a shift to the right, in contract, haemoglobin SS genotype red blood cells showed significant shift to the left after incubation in L-Arginine, Chloramphenicol and Phyllanthus amarus. In conclusion Phyllanthus amarus and Chloramphenicol (10 and 20mg/ml) and high dose L-Arginine (20mg/ml) have protecting ability against osmotic stress in PBS medium in Haemoglobin AA, AS and SS genotype red blood cells meanwhile low dose L-Arginine (10mg/ml) causes red blood cell lysis in haemoglobin AA and AS genotype red blood cells.

EFFECT OF ETHANOLIC LEAF EXRACT OF TRIDAX PROCUMBENS ON FRUCTOSE-INDUCED HYPERTENSION IN MALE WISTAR RATS: MECHANISM INVOLVED

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Fructose induces hypertension by increased cardiac oxidative stress, recent findings have also shown that *Tridax procumbens* (TP) has considerable antioxidant properties. There is however dearth of information on the effect of TP on fructose-induced hypertension. This study therefore investigated the effect of ethanolic leaf extract of TP (ELETP) on fructose-induced hypertension in male Wistar rats. Thirty-five male Wistar rats (200-250g) were used. They were randomly divided into 5 groups (n=7) and treated as follows: Group I (Control) drank tap water while groups II-V drank 10% fructose solution for 3 weeks to establish hypertension. Group I and II received normal saline, group III and IV received 100mg/kg and 400 mg/kg ELETP, respectively while group V received Prazosin (0.5mg/kg) and Propranolol (10mg/kg) orally for 4 weeks. Systolic (SBP), Diastolic (DBP), Mean Arterial Blood Pressure (MABP) and Heart Rate (HR) were determined non-invasively. Lipid profile (cholesterol, trig, HDL and LDL) were determined in plasma. Markers of oxidative stress (MDA, SOD and catalase) were determined in plasma and heart tissue. Increased SBP, DBP, MAP and HR were observed in all fructose exposed rats compared with the control. The increased SBP and DBP were lowered in groups III (136.58 ± 1.94mmHg; 96.78 ± 2.21mmHg), IV (132.17 ± 1.59mmHg; 92 ± 7.14mmHg) and V (122.95 ± 22.46mmHg; 87.56 ± 5.11mmHg) compared with group II (156.17 ± 12.09mmHg; 112.08 ± 12.90mmHg). Cholesterol, Trig and LDL levels were significantly increased in group II compared with the control. The increased Trig level in group II (140.62 ± 21.70 mg/dl) was significantly reduced in group III (100.52 ± 8.78 mg/dl). Cardiac MDA level was increased while catalase activity decreased in group II when compared with control and all treated groups. In conclusion, TP reversed fructose-induced hypertension through a mechanism that may involve its antioxidant potential.

Keywords: Fructose-induced Hypertension, *Tridax procumbens*, Oxidative Biomarkers

GREEN SYNTHESIZED TITANIUM DIOXIDE NANOPARTICLE OF *HEINSIA CRINITA* LEAVES (HC-TIO$_2$NPs) PROMOTES GASTRIC ULCERATION FOLLOWING PYLORIC LIGATION IN RATS

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Titanium dioxide nanoparticles (TiO_2NPs) are used as food additives. Previous reports showed that TiO_2NPs aggravate gastrointestinal injury. In an attempt to develop a clean, nontoxic and low-cost additive, green synthesis of titanium dioxide nanoparticles was carried out using *Heinsia crinita* leaves and its effect on Gastric ulceration induced by pyloric ligation. Twenty male Wistar rats (180-200g) were used for the study. Rats were grouped (n=5) into 4 groups1- Negative control received 0.5 ml of 1% Tween 80 (Vehicle) in distilled water; 2, 3, and 4 were the pyloric ligation-induced gastric ulcer groups which were untreated, and those pre-treated daily with 5 mg/ kg or 10 mg/kg HC-TiO_2NPs for 30 days, respectively. Gastric adherent mucus secretions were assessed in all the groups while gastric acid secretion was assessed in groups 2, 3, and 4. Gastric ulcer scores, free acidity, total acidity, and mucus secretions were evaluated using standard methods. Data were expressed as mean ± SEM and were considered significant at $P<0.05$. Total Acidity (mEq/L/4 h) increased significantly in 5 mg/kg HC- TiO_2NPs (3.11 ± 0.40) and 10 mg/kg HC-TiO_2NPs (3.58±0.24) compared to the ulcer alone group (2.04 ±0.08). Ulcer scores increased significantly in the 5 mg/kg HC- TiO_2NPs (11.56 ± 0.75) and 10 mg/kg HC-TiO_2NPs (12.94 ± 0.02) group compared to the control (3.02 ± 0.16). Gastric mucus secretion (mg/g tissue) decreased significantly in the positive control (0.11 ± 0.01), 5 mg/kg HC- TiO_2NPs (0.10 ± 0.02), and 10 mg/kg HC- TiO_2NPs (0.08 ± 0.01) groups compared to the negative control (2.67 ± 0.03). HC-TiO_2NPs eroded mucus secretion in the stomach and augmented stomach acidity thereby potentiating pyloric ligation-induced gastric ulcers in rats.

Keywords:

Heinsia crinita, Gastric ulcer, Pyloric ligation, Wistar rats, Acidity

PATERNAL ZINC DEFICIENCY DISTORTS GLUCOSE METABOLISM BY TRANSGENERATIONAL ALTERATIONS OF *DILP2* AND *DPEPCK* IN *DROSOPHILA MELANOGASTER* OFFSPRING

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Evidence exists for paternal contribution to embryogenesis and reprogramming of offspring metabolic status. Zinc deficiency is a risk factor for several metabolic outcomes that can predispose to glucose dysmetabolism, and maternal contribution to this phenomenon has been partly demonstrated. Accordingly, it is necessary to investigate whether paternal zinc deficiency alone can programme for offspring's adult life glucose dysmetabolism. Hence, the need for this study. Male adult flies that developed on zinc-deficient diet (TPEN-supplemented at 50μM and 100μM) were crossed with virgin female flies that developed on normal diet to get F1. To generate F2 and F3, F1 and F2 were respectively crossed with flies maintained on normal diet and whose parents were also maintained on normal diet. The offspring (F1-F3) were fed normal diet for seven days and sacrificed afterwards. They were thereafter assessed for changes in biochemical (glucose, trehalose, glycogen, triglycerides) and gene expression (*DILP2* and *dPEPCK* mRNA) markers of glucose metabolism. A significant increase in glucose and trehalose levels were observed in the F0 at 100μM TPEN. However, no significant difference in glucose levels was observed in F1 and F2, but a decrease in F3. Also, there was a significant decrease in trehalose levels for F1 (female), F2 (male), and F3 (male and female) but an increase in F2 (female) flies. F0 glycogen levels were also decreased, although there was no change in triglycerides. Moreover, through F1-F3, glycogen levels significantly ($p<.05$) reduced while triglycerides increased. Mechanistically, the fold change in DILP2 showed a significant increase from male parent (F0) to F2, but decreased in F3. However, *dPEPCK* mRNA increased in F0 and F3 but decreased in F1 and F2. Our findings suggest that paternal dietary zinc deficiency causes transgenerational glucose dysmetabolism partly by altering the *DILP2* and *dPEPCK* mRNA levels in *Drosophila melanogaster* offspring.

A RAT MODEL OF TORSION OF THE TESTIS

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Animal models of torsion of the testis (TT) have been used extensively to investigate the mechanisms of ischemia reperfusion injury (IRI) which invariably result from attempts to prevent its progression to necrosis. Previous models used have employed the clamping of testicular vessels with microvascular clips to induce ischemia for variable periods of time followed by removal of the clamps for reperfusion. These methods however do not adequately reflect the pathophysiological features of TT which go beyond mere occlusion and eventual restoration of blood flow to the testis. Attempts at mimicking TT by surgical twisting of the spermatic cord and its accompanying vessels to achieve ischemia while being representative of the clinical presentation have been fraught with many technical difficulties. A major technical difficulty is anchoring the

testis to the floor of the scrotum in its twisted state. Articles on the subject have not explicitly addressed these difficulties in the short descriptions encountered in the methods section. Such technical difficulties often encountered by investigators have thus resulted in inability to consistently reproduce the model among different investigators. This article provides a step by step description of the surgical induction of TT in the rat and the subsequent detorsion in order to induce IRI. The method is simple and explicit and can easily be learned and replicated by other investigators in the field.

PHYLLANTHUS AMARUS RESTORES HEPATIC AND INTESTINAL INTEGRITY BY MODULATION OF BAX/CASPASE 3 SIGNALING AND BACTERIAL TRANSLOCATION IN INTESTINAL ISCHEMIA REPERFUSION INJURY

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Oxidative stress and bacterial translocation invariably accompany intestinal ischemia reperfusion injury (IIRI) triggering systemic inflammatory response syndrome (SIRS) often culminating in multiple organ failure (MOF). Phyllanthus amarus leaf extract (PA) has gastroprotective and hepatoprotective properties. This study therefore investigated the protective effect and possible mechanism of action of PA against IIRI and hepatic injury. Fifty male Wistar rats were randomized into five groups (n = 10). The sham-operated group received 0.5 mL distilled water for seven days prior to sham surgery, while IIRI, febuxostat (FEB) + IIRI, low-dose PA (LDPA) + IIRI, and high-dose PA (HDPA) + IIRI groups underwent the experimental IIRI procedure. IIRI, FEB + IIRI, LDPA + IIRI, and HDPA + IIRI received 0.5 mL of distilled water, 10 mg/kg of febuxostat, 200 mg/kg of PA, and 400 mg/kg of PA, respectively, for seven days prior to the IIRI procedure. Malondialdehyde, nitric oxide,TNF-_, IL-6, and myeloperoxidase activity, reduced glutathione, thiol and non-thiol proteins, and superoxide dismutase, catalase, and glutathione peroxidase activities in intestinal and hepatic tissues. Bacterial translocation was assessed by colony counts of cultured hepatic homogenate. Bax/caspase 3 signaling by immunohistochemical staining, intestinal and hepatic histoarchitecture by H&E staining. Administration of PA attenuated IIRI-induced rise in intestinal and hepatic injury markers, malondialdehyde, nitric oxide,TNF-_, IL-6, and myeloperoxidase activities. In addition, PA reversed IIRI-induced suppression of reduced glutathione, thiol and non-thiol proteins, and superoxide dismutase, catalase, and glutathione peroxidase activities in intestinal and hepatic tissues. I/R-induced bacterial translocation was suppressed along with downregulation of I/R-induced activation of Bax/caspase 3 signaling. There was reversal of I/R-induced distortion of intestinal and hepatic histoarchitecture. PA exerted a protective effect against IIRI-induced hepatic injury by suppressing bacterial translocation and oxidative stress-mediated activation of Bax/caspase 3 signalling. These effects may be ascribed to its constituent bioactive tannins, anthocyanin, alkaloids, and phenolics.

POSTISCHEMIC ADMINISTRATION OF FEBUXOSTAT AND VITAMIN E AMELIORATES TESTICULAR ISCHEMIA- REPERFUSION INJURY IN RATS BY SUPPRESSION OF OXIDATIVE STRESS AND INFLAMMATION.

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The pathway of ischemia reperfusion injury (IRI) has been shown to involve generation of reactive oxygen species (ROS) during ischemic phase and in the immediate reperfusion phase. This study investigated the effect of blockage of sources of ROS in the ischemic and reperfusion phases of testicular-IRI. 30 male Wistar rats (180-200g) were grouped (n=6): 1, Sham operated (SO) ;2,Torsion+Detorsion(TD);3,Torsion+FEB+Detorsion(TFD);4,TD+Vit.E(TDVE);5,TFD+VE(TFDVE).Group 3,4 and 5 received 5mg/kg of FEB after 30minute of surgically induced TT for 1 hour, 10mg/kg of VE 30minutes after detorsion and 5mg/kg of FEB after 30minutes of TT+10mg/kg of VE 30minutes after detorsion respectively via i.p. Blood samples and tissues were collected after 3 days of detorsion. Tissue GPx, GSH, total thiol, SOD, MDA, XO, MPO was done. Serum NO, TNF-α, IL-1β, LH, FSH, inhibin and testosterone were estimated. Semen analysis and sperm DNA damage was assessed from the caudal epididymal fluid. Histology of the testes was also assessed. Data was recorded as mean±SEM. Statistical significance was set at $p<0.05$. The significant ($P<0.05$) increase in XO and MDA but reduction in SOD, CAT, GSH, protein and non-protein thiols in TD group was reversed significantly in TFD than TDVE and TFDVE.TFD group mostly reduced inflammatory mediators which were raised in TD group. Testosterone level was raised in TFD and TFDVE groups. Increased sperm DNA damage and reduced sperm indices were observed in TD group which were reversed in TFDVE and TFD than TDVE. There was an improvement in testicular cytoarchitecture in TFDVE group. Blockage of Xanthine oxidase in the ischemic phase with febuxostat and ROS burst after reperfusion with Vitamin E after TT onset may offer a viable and practical alternative in the treatment of torsion of testes. Blocking other sources of ROS may help to reduce testicular-IRI

COMPARATIVE ANALYSIS OF THE EFFECT OF AQUEOUS EXTRACT PHYLLANTHUS AMARUS, L-ARGININE AND CHLORAMPHENICOL ON OSMOTIC FRAGILITY ON RED BLOOD CELLS OF DIFFERENT GENOTYPES (HBAA, HBAS AND HBSS)

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Red blood cells of different haemoglobin genotypes (HbAA, HbAS and HbSS) was incubated in *Phyllanthus amarus*, chloramphenicol and L-arginine after which the red cells were subjected to osmotic stress test in phosphate buffered saline. The aim of the experiment was to ascertain/compare the effect of Phyllanthus amarus aqueous extract, L-arginine and chloramphenicol on the integrity of red cell membranes of the different genotypes. Doses of 10 mg and 20 mg/ml of aqueous extract of Phyllanthus amarus,

Chloramphenicol and L-Arginine was used for the study. Washed red cells were incubated in each of the drugs/extract for 3 hours before subjecting to osmotic stress in serial dilutions of PBS, for 30 minutes, spun in a bucket centrifuge at 2500 rpm for 10 minutes. Absorbance of the supernatant was measured using a spectrophotometer at 540nm wavelength. Results of percentage haemolysis was analysed against serial dilutions of PBS. Result showed a shift to the left in haemoglobin AA genotype cells incubated in 10mg and 20mg Phyllanthus Amarus, 10mg and 20mg chloramphenicol, 20mg L-Arginine but a shift to the right in cells incubated in 10mg L-Arginine. Same was recorded for Haemoglobin AS genotype red blood cells except for 10mg and 20mg L-Arginine which showed a shift to the right, in contract, haemoglobin SS genotype red blood cells showed significant shift to the left after incubation in L-Arginine, Chloramphenicol and Phyllanthus amarus. In conclusion Phyllanthus amarus and Chloramphenicol (10 and 20mg/ml) and high dose L-Arginine (20mg/ml) have protecting ability against osmotic stress in PBS medium in Haemoglobin AA, AS and SS genotype red blood cells meanwhile low dose L-Arginine (10mg/ml) causes red blood cell lysis in haemoglobin AA and AS genotype red blood cells.

SALT SENSITIVITY, SYSTEMIC AND VASCULAR INFLAMMATION IN NORMOTENSIVE YOUNG-ADULT NIGERIANS

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High salt diet is the most important environmental risk factor for hypertension (1), globally, about 50% of essential hypertension tagged essential are salt-sensitive (2). about 56% and 34% adult hypertensive and normotensive Nigerians are salt–sensitive (3). Recently an increase in the prevalence of children and adolescent hypertension has been reported (4). To prevent the development of salt-sensitive hypertension, the search for the predictors of salt sensitivity becomes imperative. This study was designed to demonstrate the association between blood pressure, salt sensitivity and systemic and vascular inflammation in normotensive young-adult Nigerians. Recruited participants are consenting normotensive (Systolic < 90), young adults (18-35 years of age) without any known underlying pathology. 12-hour nocturnal urine and baseline parameters (anthropometric parameters, blood samples) were collected/ determined on day 1 of the experiment, participants were given a salt-load at a dose of 200mmol/day Na+ for 5 days. Blood pressure parameters were measured before and after salt-loading using a non-invasive electronic Omron M6 BP monitor which has been calibrated against an Accoson mercury sphygmomanometer. Salt Sensitivity was determined as >5mmHg difference in MABP of participants post salt-loading. Plasma concentration of biomarkers of systemic and vascular inflammation (CRP, Il-1b. IL-6, Il-17, MIP-1 TNF-a, VCAM-1 and ICAM-1) were estimated before and after salt-loading. High salt diet elevated BP in the salt sensitive participants. 27% of the participants are salt sensitive (SS). Plasma concentration of IL-6 is significantly lower in SS participants compared to SR counterparts before and after salt loading; while IL-17, CRP and VCAM-1 concentrations are significantly elevated in SS participants before salt loading. The plasma CRP and IL-6 concentrations of female SS participants are significantly higher. In a young-adult normotensive Nigerian population, it appears that IL-1b, IL-6 and VCAM-1 are predictors of salt sensitivity and future salt sensitive hypertension. However, the mechanism behind this phenomenon needs to be unravelled.

ANTI-OXIDATIVE AND ANTI-INFLAMMATORY EFFECTS OF VIRGIN COCONUT OIL AGAINST TRICHLOROACETIC ACID ASSAULT ON THE LIVER, STOMACH AND COLON OF RATS

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Trichloroacetic acid (TCA), a major end-metabolite of trichloroethylene and tetrachloroethylene which humans are exposed to occupationally and environmentally, is a toxicant and chemical carcinogen. It causes organ damage via excessive generation of free radicals, depletion of antioxidant system and activation of pro-inflammatory cytokines. We explore the mechanisms underlying the anti-oxidative and anti-inflammatory mechanisms of Virgin Coconut oil (VCO) alone or combined with 5-fluorouracil (5FU) in the treatment of TCA-induced hepatic, gastric and colonic damage in the rat. Rats were randomly assigned to seven groups (n=5) viz; Group 1: 1mL/day Normal Saline (NS), Group 2-7: TCA (250 mg/Kg b.wt, p.o) for ten days, followed by VCO and 5FU treatment for another ten days except the Group 2 which served as control. Group 3: 5% of VCO per gram of feed+TCA, Group 4: 10% of VCO per gram of feed+TCA, Group 5: 5-FU (50 mg/kg, i.p) + TCA, Group 6: 5% of VCO per gram of feed + 5-FU (50 mg/kg, i.p) + TCA, Group 7: 10% of VCO per gram of feed + 5-FU (50 mg/kg, i.p) +TCA. Serum liver enzymes, tissue oxidative stress parameters, inflammatory markers were evaluated along with histological examination. TCA's elevation of serum transminases (ALT, AST) and alkaline phosphatase (ALP), as well as Total Bilirubin (T.Bil), level was abrogated by VCO either alone or in combination with 5-FU in a dose-dependent manner. Similarly, decreased activity of SOD, CAT, GPx and Nrf2 in the liver, stomach and colon were enhanced, while MDA, MPO, TNF-α, IL-1β and NF-kB level hitherto increased by TCA were lessened by VCO. Further, histomorphometry data in the stomach, colon and liver favoured the anti-oxidative and anti-inflammatory potentials of VCO observed in the study. Virgin coconut oil lessens trichloroacetic acid induced oxidative stress and inflammation in the liver, stomach and colon.

NONI FRUIT (*MORINDA CITRIFOLIA*) PRODUCES ANTI-OXIDANT AND ANTILIPEMIC EFFECT AGAINST BISPHENOL-A INDUCED CARDIAC TOXICITY IN FEMALE WISTAR RATS.

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Bisphenol A is an environmental degradation agent and chemical used in manufacturing and food industry with a mainstay of downstream regulation. Noni on the other hand is a widely consumed dairy products of Pantropical origin. This study therefore aimed to examine the ameliorating effect of aqueous extract of Noni fruits on Bisphenol-A induced cardiotoxity and its associated metabolic risk factors in male Wistar rats. Twenty-four (24) male Wistar Rats weighing 120±50g were divided into four (4) groups of six rats (6); n=6, after a week of acclimatization as: Control (Ctr: normal chow + vehicle: Olive oil), Bisphenol A (7g/kg, orally), Noni aqueous extract (NAE:500 mg/kg; orally), and NAE:500mg/kg+ Bisp A;7g/kg. After six weeks of experimental procedure, each animal was anesthetized with 0.8mg Phenobarbitone and blood sample was collected by cardiac puncture. Plasma and cardiac tissue homogenate were analyzed for biochemical parameters and data were expressed as mean ± SEM and p-values < 0.05 were accepted as significant. Bisphenol A reduced significantly plasma and cardiac histone deacetylate (HDAC), adenosine and Nitric oxide (NO) while causing a significant increase in triglycerides (TG), lipoprotein A, tumor necrotic factor alpha (TNF-α), uric acid (UA), catalase and **(**malondialdehyde) MDA. The aqueous extract of Noni on the other hand increased significantly HDAC and NO while reducing significantly TNF-α, catalase, MDA, TG, and Lipoprotein A with a marginal decrease in UA. From the study, the NAE reduced worsening cardiovascular risk factors while causing a positive epigenetic expression through improved HDAC and neovascularisation activities.

Keywords: HDAC, cardiovascular risk factors, Noni aqueous extract, Bisphenol A, Wistar rats

AMELIORATIVE EFFECT OF VITAMIN E AND N-ACETYLCYSTENINE ON LEAD ACETATE-INDUCED GLUCOSE METABOLISM IMPAIRMENT IN WISTARS RATS

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Lead exposure has been reported to induce disarray in glucose metabolism majorly by tissue oxidative damage. Vitamin E (VitE) and/or N-acetylcysteine (NAC) have been documented to ameliorate lead toxicity. Data on the effect of vitE and/or NAC on lead-induced impairments of glucose metabolism are scanty. This study therefore investigated the effect vitE and/or NAC on glucose metabolism in lead acetate exposed rats. Forty male Wistar rats(100-140g) grouped into 5 (G1-5) were treated as follows; G1 received the vehicle only, G2-5 received lead acetate (20mg/kg, p.o) while G3-G5 were orally treated with VitE (150mg/kg), NAC (100mg/kg) or a combination of both, respectively for 30 days. Oral glucose tolerance test (OGTT) was carried out and Area under the curve (AUC) was calculated. Fasting blood glucose (FBG), Insulin, lactate, Lactate Dehydrogenase activities (LDH), HOMA-IR, oxidative stress biomarkers (MDA, SOD, Catalase and GPx) and liver function test (ALT, ALP and AST) were determined in the blood. Hepatic glycogen content was determined using the anthrone method. Data are presented as Mean±SEM, analysed by ANOVA at P<0.05 followed by TuKey posthoc test. Lead exposure caused significant increased FBG (101.14 ± 6.10mg/dl), HOMA-IR (0.189±0.17) and plasma lactate level (32.75±8.44mg/dl) in G2 relative to G1 (79.25± 2.60mg/dl, 0.014± 0.003, 29.04±0.12mg/dl). The increase FBG and HOMA-IR were significantly decreased by vitE or/and NAC in G3, G4 and G5 respectively, relative to G2. Hepatic glycogen content was significantly depleted in G2 and reversed in G3-G5 relative to G1. Increased AST and ALT levels were observed in G2 relative to control and the treated groups. Although, MDA level was not different across all groups, GPx and catalase activities were significantly reduced in G2 and reversed in G3-G5 relative to control. Data from this study indicate that vitamin E and N-acetylcysteine prevented lead-induced glucose impairment by potentiating the endogenous antioxidant activities.

Keywords:

Lead toxicity, Glucose metabolism, Vitamin E, N-acetylcysteine

OXIDATIVE STRESS INDUCED BY RESTRAINT, MIRROR AND INTRUDER STRESSORS ALTERS CREATININE, UREA, AND RENAL TUBULOGLOMERULAR MEMBRANE ANTIOXIDANT INTEGRITY IN RATS

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The pervasive nature of the global economic meltdown has made stress a general phenomenon and is believed to contribute to various disease conditions. The study examined effect of oxidative stress on creatinine, urea, and renal tubuloglomerular membrane antioxidant integrity in Female Wistar rats. 24 adults female Wistar rats weighing 160-200g and within the ages of 12-14weeks were used for experiment 1, while 12 offspring were utilized for experiment 2. Kidney tissues were isolated from the animal and homogenized for antioxidant assay. Serum was collected for assays of Urea and creatinine using ELISA. Data collected was analyzed for Mean±SEM and One Way ANOVA. Our results revealed that the different stressors reduced relative kidney weights, but did not significantly alter serum creatinine concentration, however, the concentrations were slightly increased compared to control.

Urea concentration was significantly increased in rats exposed to restraint and intruder stressors. Exposure to mirror stressor did not alter urea concentration. Offspring of stressed female Wistar rat exhibited significant increase in serum urea level, minimal increase in serum creatinine levels. Markers of oxidative stress revealed that GSH, GST, GPx, SOD, MDA and CAT were altered depending on the stressor applied. Exposure to restraint stressor decreased the activities of GPx, SOD and CAT in the kidney of the rats. Exposure of the rats to mirror stressor decreased the activities of GPx and CAT in kidney, while increasing the activity of kidney SOD. When the rats were stressed by exposure to intruder stressor, it decreased the activities of kidney GPx and SOD, but it also increased the activity of kidney SOD. Kidney MDA levels were increase irrespective of the stressor applied. In all, continued exposure of the rats to stressful condition has the tendency of compromising the integrity of liver and kidney function, thus, with potency of compromising female reproductive outcome.

Keywords: Oxidative Stress, Antioxidants, Creatinine, Urea, Kidney

MECHANISTIC EFFECTS OF CAFFEINE CONSUMPTION ON REPRODUCTIVE FUNCTIONS OF FEMALE WISTAR RATS

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Caffeinated beverages are widely consumed and have been implicated in several bodily dysfunctions. Chronic exposure to caffeine has been linked with decreased female fertility. This study assessed the effects of caffeine consumption and its withdrawal (recovery) on reproductive functions of female Wistar rats. Thirty-five adults female Wistar rats were divided into seven groups (n=5); Group I - control (distilled water), II-1V received 10, 20 and 40 mg/kg/day of caffeine orally for 21 days respectively, V-VII received 10, 20 and 40 mg/kg/day of caffeine respectively for 21 days and allowed to recover for another 21 days. Rats were sacrificed and ovaries, fallopian tubes and uteri were harvested for evaluation of Malondialdehyde (MDA), Nitric oxide (NO), reduced Glutathione (GSH) levels, Superoxide dismutase (SOD) and Catalase activities by spectrophotometry. Serum Luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol levels were measured by ELISA, and organ histology was observed microscopically. Data were analyzed using ANOVA and $p<0.05$ was considered statistically significant. Caffeine increased MDA, NO and catalase activity of ovaries, fallopian tubes and uteri in a dose dependent manner, which were reduced upon withdrawal. Caffeine reduced GSH level of ovary and fallopian tubes which increase after its withdrawal. Serum LH was reduced during caffeine withdrawal from 20 and 40mg/kg/day, FSH reduced in 40 mg/kg/day while estradiol reduced during treatment in a dose-dependent manner when compared with control. Caffeine caused dose dependent alterations in the architecture of the ovaries via congested connective tissues, sloughed plicae of muscularis of fallopian tubes and degenerated epithelial layer of the uteri with severe infiltration of inflammatory cells in the stroma of its myometrium, conditions which remain during caffeine withdrawal. The study showed that caffeine consumption may adversely alter the reproductive functions of female Wistar rats.

Key words: *Caffeine, Infertility, Oxidative Stress, Reproductive hormone*

METHANOLIC EXTRACT OF *Ricimus communis* LEAVES AMELIORATES DICHLORVOS-INDUCED NEPHROTOXICITY IN MALE WISTAR RATS

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2-2-dichlorovinyl dimethyl phosphate (DDVP/Dichlorvos) causes nephrotoxicity (Yadav *et al*, 2012); a major health challenge with high mortality rate due to its expensive management. The search for a potent, safe and less expensive medicine to treat nephrotoxicity led to the use of herbal-based medicine. The study investigated the effect of *Ricinus communis* (RC) on DDVP-induced nephrotoxicity. Thirty-two (32) male rats (200-250g) were randomly divided into four (n=8) groups. Group I (Control) received 10ml/kg of dimethyl sufoxide and distilled water solution (vehicle) for six weeks; Group II were exposed to DDVP (1ml) via inhalation for 4 weeks ; Group III were exposed to DDVP and then administered with 300 mg/kg RC while Group IV received only RC (300mg/kg) orally for six weeks. After treatment, the animals were euthanized; blood was collected via cardiac puncture into EDTA bottles and centrifuged to obtain plasma for electrolytes. The kidneys were excised and used for histology, immunohistochemistry, estimation of oxidative stress and inflammatory markers and DNA fragmentation test using standard methods. DDVP caused significant increases ($P<0.05$) in Na+, creatinine, urea, urinary protein, inflammatory markers, MDA and DNA fragmentation while RC reduced ($P<0.05$) elevated levels of renal and inflammatory markers, MDA and DNA fragmentation. The results suggest that *Ricinus communis* extract possesses nephro-protective properties.

ETHANOLIC SEED EXTRACT OF *MACUNA PRURIEN* AMELIORATED XEROSTOMIA IN ROTENONE-INDUCED PARKINSONISM IN RATS

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Parkinson's disease (PD) is a chronic, progressive, and irreversible neurodegenerative disorder with increasing prevalence worldwide and reported reduced saliva flow and composition in Parkinsonism. *Macuna Prurien* seed is used in the management of PD locally. This study was designed to investigate the effect of ethanolic seed extract of *Macuna Prurien* on saliva and its composition in rotenone-induced parkinsonism in male rats.

Thirty-two (32) male Wistar rats weighing between 200-220g were randomly grouped into four groups consisting of eight (8) rats per group. Parkinsonism was induced with a daily oral administration of Rotenone 1mg/kg for 7 days. The control group received 1 mg/kg of olive oil (vehicle), the Rotenone alone group (1mg/kg of rotenone with no treatment), and groups 3 and 4 received 1mg/kg of rotenone and were treated with 50mg/kg and 100mg/kg of the *Macuna Prurien* seed extract daily for 28 days, respectively. Behavioural studies were conducted to ascertain parkinsonism. Saliva was collected for flow rates, pH, biochemical assays of relevant enzymes, and electrolyte determination, using standard procedures. All data were expressed as mean ± standard error of the mean (SEM), and statistical analysis was conducted using ANOVA and significant accepted at $P<0.05$. The 100 mg/kg and 50 mg/kg groups showed a significant increase in the salivary volume and flow rate. There is no significant change in the pH of the saliva and the electrolyte of the saliva. Groups with the test extracts (100 mg/kg and 50 mg/kg rotenone) showed decreases in the salivary amylase content compared to Rotenone alone group. The ethanolic extract of *Macuna prurien* seed caused an increase in the volume and rate of salivary secretion following the induction of parkinsonism, thus ameliorating the silenced xerostomia in parkinsonism.

Keyword: Parkinsonism, Rotenone, *Macuna prurien* extract, Salivary rate

USE OF FACEMASKS IN COVID-19 AND CORONA VIRUS PROTECTION AND STRESS PERCEPTION AMONG A NIGERIAN POPULATION

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COVID-19 pandemic made the use of face masks mandatory globally as a measure for minimizing the transmission risk of the virus. Amidst potential poor masking compliance, it is unclear if masking discomfort is related to type of masking. These masks may also impose physiological and psychological burden on healthy persons. We investigated the perception of stress symptoms in subjects using various face masks. The study was a cross-sectional design using questionnaires among healthy individuals in Mushin and Surulere LGAs. Data are presented as frequency and percentage in numeral, and analysis for association evaluated with chi-square test. A total of 541 volunteers participated in the study, including 302 (55.8%) males and 239 (44.2%) females. They were mostly young adult around 18-29yrs (50.8%), followed by those in 30-39yrs (19.5%), 40-49yrs (15.4%), 50-59yrs (10.7%) and ≥60yrs (3.7%). Nearly all the participants were educated (99.28%), with basic (41.7%) and post-basic (58.3%) education. 67.7% of the participants were working, while 32.3% were not working. The most frequently used mask was disposable surgical mask (85.6%), with other masks such as cotton, Ankara, N95 and polyester/silk at 5.7%, 3.4%, 2.8% and 2.2% respectively. The most common stress symptoms associated with face masking included uneasiness to eat and drink (71%), stuffiness (43.3%), communication barrier (39.6%) and breathlessness (38.6%). The least experienced stress symptoms were fatigue (22.6%), skin irritation (21.6%) and headache (15.7%). Significant association existed between mask type and stress indices such as headache ($P<0.01$), tightness ($P<0.01$) and general discomfort ($P<0.01$). Disposable Surgical mask was the most used among Lagos residents during the pandemic, more people were generally uncomfortable, with no age/gender-related associated discomfort.

Keywords: *face mask, discomfort, covid-19, age, gender*

SULFASALAZINE PROTECTS AGAINST THE WORSENING EFFECT OF ROTENONE ON LIVER AND COLONIC DAMAGE IN ACETIC ACID INDUCED COLITIS IN RATS

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Drugs used to treat colonic inflammation have been mentioned as possibly having an impact on Parkinson's disease (PD) since scientific data show PD associated neuroinflammation is driven by the inflammation of the colon. This study examines the effect of rotenone and sulfasalazine on liver enzymes and colon of rats with colitis. Adult male Wistar rats were randomly divided into five. Group A (control) received a vehicle, Group B received rotenone only. Groups C and D were given 1ml of 4% acetic acid intrarectally to induce colitis, after which Groups D and E were administered 500mg/kg sulfasalazine orally for 14 days, either after colitis induction or alone respectively. Administration of intraperitoneal infusion of 2.5 mg/kg rotenone commenced for groups B to E by day 7 of sulfasalazine treatment for 14 days. Rats were sacrificed on days 7, 14 and 21. Blood samples were collected for liver enzyme determination and the distal colon was taken for biochemical analysis of oxidative stress markers. By day 14 rotenone increased liver enzymes (ALP and ALT), colon myeloperoxidase (ng/mg protein): 213.11± 80.9, 167.33± 14.8 in groups B and C compared to control (99.3±26.4). Superoxide dismutase (mmol/mg protein) decreased in groups B to E 21.62± 1.23; 18.73±0.82; 17.37±0.90; 20.90± 0.10 respectively compared with control (27.81± 0.82) ($P< 0.001$), There were decreases in reduced glutathione ($P< 0.05$), sulfhydryl level and Na+/K+ ATPase activity in the colonic samples of groups B and C compared to the control. Sulfasalazine lowered MPO, boosted colonic levels of the antioxidant enzymes and the pump activity and also decreased liver enzymes when compared with the control thereby ameliorated rotenone effect. The anti-oxidant and anti-inflammatory properties of sulfasalazine may be responsible for the observed protection which could be helpful in preventing the development of Parkinson's disease.

Keywords

Sulfasalazine, colon inflammation, rotenone, liver enzymes

MODULATORY EFFECTS OF CONCURRENT TREATMENT OF CANNABIDIOL (CBD) ON GLUCOSE AND LIPID METABOLISM IN RITONAVIR AND HIGH FAT DIET TREATED ADULT MALE ALBINO WISTAR RATS

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HIV-related lipodystrophies are the most common form of lipodystrophy affecting up to 50 % of HIV patients, owing to the use of protease inhibitors in HIV treatment. Cannabidiol (CBD) has been reported to positively affect metabolic and nervous functions; it is however not clear whether CBD can ameliorate the lipodystrophic effects induced by protease inhibitor (PI). The present study was thus designed to evaluate the modulatory effects of 2 weeks concurrent treatment of CBD on lipid and glucose metabolism in protease inhibited adult male albino Wistar rats. Adult male wistar rats weighing 180-200 g were divided into five groups (n=7). Group 1 animals served as control and were untreated. Group 2-5 were treated with protease inhibitor and high fat diet (HFD) for 2 weeks. Concurrent treatment of apple cider vinegar (0.8 mg/Kg), CBD (10 and 25 mg/kg) were administered to groups 3-5 animals respectively. Glucose and lipid metabolic parameters (such as OGTT, ITT, lipid profile and fasting serum insulin levels), hepatic oxidative stress markers, liver function test and hepatic leptin/JAK 2 expressions were determined. 2 weeks treatment of rats with ritonavir and HFD caused lipodystrophy like symptoms evidenced by hepatic steatosis, hypercholesteremia, impaired liver function, high glucose intolerance and reduced insulin sensitivity. Concurrent treatment of animals with CBD mitigated the ritonavir and HFD induced alteration in glucose and cholesterol metabolism which was associated with improved insulin response and increase in hepatic leptin expression. The beneficial effects of CBD treatment on lipodystrophy appears to involve the suppression in the c-Jun N-terminal kinase (JNK) pathway as animals treated with CBD in the present study had lowered lipid peroxidation and decreased JAK-2 hepatic expression.

Keywords: Lipodystrophy, Metabolism, Cannabidiol, JAK-2 pathway

MITIGATING EFFECT OF SULFASALAZINE ON ULCERATIVE COLITIS TRIGGERED OXIDATIVE STRESS IN REGIONS OF RAT BRAIN.

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Ulcerative colitis is a gastrointestinal dysfunction believed to cause inflammation in the brain in a caudo-cranial fashion. This study investigated the effect of acetic acid (AA) induced colitis and sulfasalazine treatment on biomarkers of oxidative stress in regions of rat brain. Male Wistar rats with the average weight of 140g were randomly divided into four groups. Group A (control) was given normal saline, groups B and C were induced with colitis by single intrarectal administration of 1ml of 4% AA. Group C and D were treated with 500mg/kg sulfasalazine orally for 14 days either after colitis induction or alone respectively. On day 3, 7, 14 and 21 after colitis induction, rats were sacrificed with the brain removed for gross examination while indicators of oxidative stress in the striatum, cortex and cerebellum of rat brains were assayed. Results showed colitis increased NO and H_2O_2 in the brain regions analysed all through the study while MDA significantly increased by day 14 except in the cerebellum. SOD, GSH and Na^+/K^+ ATPase activity decreased in the brain regions of the AA group compared with control. Sulfasalazine ameliorated the effects of colitis on the brain by dousing the pro-oxidant and stimulating the anti- oxidant enzymes. Colitis triggered prolonged oxidative stress in the brain which suggests the effect of gastrointestinal inflammation goes beyond peripheral effect. Sulfasalazine protected the brain against the effect of colitis.

Keyword: Acetic acid, Colitis, inflammation, brain, sulfasalazine

METHANOL EXTRACT OF *PARQUETINA NIGRESCENS* (AFZEL.) BULLOCK LEAF (MEPL) AND SQUALENE AMELIORATE ARSENIC TRIOXIDE-INDUCED REPRODUCTIVE TOXICITY IN MALE WISTAR RATS

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The concentration of arsenic in Nigeria's groundwater, which is the major source of drinking water is high. Arsenic causes male reproductive dysfunction by inducing oxidative stress. *Parquetina nigrescens* leaf, reported to have antioxidant property, is used in African traditional medicine to improve sexual performance. This study was designed to investigate and compare the effects of methanol extract of *Parquetina nigrescens* leaf (MEPL) and squalene on gonadal functions in arsenic trioxide-treated (As_2O_3) male Wistar rats. The MEPL and analytical grade squalene were used for the study. Forty male Wistar rats (150-180 g), divided into 8 groups (n=5), were treated orally for 54 days as follows: group 1 (10% tween 80), group 2 (3 mg/kg As_2O_3), group 3 (250 mg/kg MEPL), group 4 (500 mg/kg MEPL), group 5 (100 mg/kg squalene), group 6 (As_2O_3+250 mg/kg MEPL), group 7 (As_2O_3+500 mg/kg MEPL), group 8 (As_2O_3+100 mg/kg squalene). Epididymal sperm, testicular malondialdehyde, glutathione peroxidase, 17-beta hydroxysteroid dehydrogenase (17β-HSD), serum testosterone, 8-oxo-2-deoxyguanosine (8-OHdG), testicular Bax, Bcl-2

expression, and testicular tissues were evaluated. Data were analysed using ANOVA at $\alpha_{0.05}$. Sperm motility increased while the percentage of abnormal sperm morphology decreased in As_2O_3+250 mg/kg MEPL, As_2O_3+500 mg/kg MEPL and As_2O_3+squalene treated groups when compared with As_2O_3. Testosterone level, glutathione peroxidase, 17β-HSD, and the expression of Bcl-2 increased while testicular malondialdehyde, 8–OHdG, and Bax expression decreased in As_2O_3+250 mg/kg MEPL, As_2O_3+500 mg/kg MEPL and As_2O_3+squalene treated groups compared with As_2O_3. Atrophy of seminiferous tubules, depletion of germ cell layers, and absence of spermatozoa were observed in the As_2O_3 group, but not in groups co-treated with MEPL or squalene. Methanol extract of *Parquetina nigrescens* leaf and squalene ameliorated arsenic trioxide-induced gonadal toxicity *via* the prevention of cell death and oxidative stress in male rats.

Keywords: *Parquetina nigrescens*, Squalene, Testicular toxicity, Arsenic trioxide, Apoptosis

BODY WEIGHT PERCEPTION AND ITS RELATIONSHIP WITH ANTHROPOMETRIC INDICES OF UNDERGRADUATE STUDENTS IN PORT HARCOURT, NIGERIA

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Influence from the media has increased the consciousness of young adults about their body weight and size. The aim of the present study was to evaluate body weight perception and its relationship with anthropometric indices of undergraduate students in Port Harcourt. The study involved a total of 600 undergraduate students aged 18-35 years (including 249 males and 351 females). Body weight perception was assessed using a structured questionnaire. The anthropometric indices (body mass index, waist, hip and shoulder circumferences and foot length) of each subject were measured using standard methods while the ratios were calculated. The average BMI of undergraduates was 23.66±0.13kg/m^2. There was no significant gender difference in BMI, hip circumference and foot length. Waist circumference and waist-to-hip ratio were significantly higher in males while waist-to-height ratio was significantly higher in females. There was significant correlation between BMI and hip circumference, shoulder circumference and waist circumference but no significant correlation exists between BMI and foot length. The actual incidence of obesity amongst undergraduates in Port Harcourt was 3.3% using the measured BMI even when only 1.5% perceived admitted being obese. The actual BMI classified overweight was 25.7% as against perceived overweight of 19.8%. Only 56.3% perceived themselves to be within the normal weight whereas up to 67.5% were actually normal weight. The actual incidence of underweight was 3.5% but as many as 22.3% of undergraduates perceived they were underweight. In conclusion, there is a similarity between actual BMI and perceived BMI amongst undergraduates. Using both the actual and perceived BMI classifications, more of the males were within the underweight and normal weight classes whereas overweight and obesity were commoner in the females. Although there was gender similarity in BMI, waist-to-height ratio was significantly higher in females whereas waist-to-hip ratio was significantly higher in males. Waist-to-height ratio therefore was more useful in assessing abdominal size in females while waist-to-hip ratio was more useful in assessing abdominal size in young adult males. Foot length was the poorest tool in assessing body weight.

VANADIUM MODULATES NA$^+$-K$^+$ATPase AND SODIUM-GLUCOSE-CO-TRANSPORTER 1 ACTIVITIES IN NORMAL AND DIABETIC RATS.

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A major function of the gastrointestinal tract is transport of glucose across the membrane of small intestine via sodium-glucose-co-transporters (SGLT) in a process driven by Na$^+$-K$^+$ATPase. Diabetes is a disease of global concern, with uncontrolled gut glucose absorption, which is a major determinant. The production of α-glucosidase inhibitors targeted at reducing absorption of gut macronutrients has been beneficial though with little setbacks. Vanadium has been reported as a potential oral therapeutic adjunct in diabetic control but its mechanism of action on intestinal glucose uptake is unclear, which this study investigate in normal and diabetic rats. Thirty male wistar rats were divided to 6 groups (n=5): Group I served as negative control, II and III received 20mg/kg/p.o, and 40mg/kg/p.o of Na_2VO_3 respectively while the other groups were induced with diabetes (Streptozotocin, 65mg/kg/i.p) without (Group IV) and with exposure to 20mg/kg/p.o (Group V) and 40mg/kg/p.o (Group VI) of Na_2VO_3 for 8week. Body weight (measured daily), blood glucose level (BGL measured using glucose oxidase method), intestinal glucose absorption (measured using everted sac method), intestinal tissue Na$^+$-K$^+$ATPase pump activity (measured spectrophotometrically), expression of SGLT1/SLCA51 gene were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. Sodium-metavanadate significantly reduced body weight of Streptozotocin-induced-diabetic groups and BGL of both diabetic and normal rats. Rate of glucose absorption significantly decreased in Na_2VO_3 groups compared with negative-control and in the diabetic Na_2VO_3 groups compared with positive-control. Na$^+$-K$^+$ATPase activity was significantly decreased in 40mg/kg Na_2VO_3 group compared with negative-control and in diabetic+20 and 40mg/kg Na_2VO_3 groups compared with positive-control. Expression of intestinal SGLT1 was downregulated in Na_2VO_3 rats relative to positive-control. Vanadium treatment in normal and Streptozotocin-induced diabetes decreased blood glucose level and rate of glucose absorption

through inhibition of Na^+-K^+ATPase activities resulting in a decreased expression of intestinal SGLT1 gene.

Keywords: Sodium metavanadate (Na_2VO_3), Diabetes and Na^+-K^+ATPase activity, sodium-glucose-co-transporter 1 (SGLT1) expression

EFFECT OF GINGER *(ZINGIBER OFFICINALE)* AND MARIJUANA *(CANNABIS SATIVA)* ON FEAR AND ANXIETY IN SWISS MICE.

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The effects of consumption of powdered marijuana diet and ginger diet on fear and anxiety were studied using Swiss mice. The mice were randomly assigned into three groups. Group 1 served as control and was fed with normal rodent chow. Group 2 was fed with ginger diet (5g of powdered ginger mixed with 95g of rodent chow, making 5% of ginger diet) while group 3 was fed with marijuana diet (5g of powdered marijuana mixed with 95g of rodent chow, making 5% of marijuana diet). Feeding was allowed for 14 days before the experiment started. The animals had free access to their feeds and water. Food and water intake were measured daily. The Elevated Plus-Maze was used to assess fear and anxiety in the mice.

Results showed that 5g ginger diet had no significant effect on the animal behaviour in terms of time spent in the light and dark fields as well as the time spent in grooming while 5g marijuana diet significantly increased time spent in light and decreased time spent in dark and time spent grooming. These alterations in the marijuana group indicate a reduction in fear and anxiety. Consumption of 5% marijuana diet suppressed fear and anxiety in Swiss mice while consumption of 5g ginger did not affect fear and anxiety in these animals.

Keywords

Zingiber officinale (Ginger),Cannabis sativa (Marijuana), Fear and Anxiety.

ANTI-COLITIS POTENCIES OF POTASSIUM BROMATE ($KBrO_3$) AT THERAPEUTIC DOSE IS ASSOCIATED WITH HEPATOPROTECTION AND OSTEOHOMEOSTASIS

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Potassium bromate ($KBrO_3$) has been considered as a dose-dependent bifunctional compound but toxic at high doses. Although, its anti-gastric ulcer healing has been reported, we recently showed that it ameliorated Crohn's colitis healing. In this multi-phased study, we evaluated the effect of $KBrO_3$ on osteological and hepatic functional changes as extra-intestinal organs associated with wound healing during Crohn's colitis.

60 male Wistar rats (180-200g) were divided into 6 groups (n=10), CON-control, CU-Crohn's colitis untreated, CV-Crohn's colitis treated with vitamin E, CK-Crohn's colitis + potassium bromate, CVK-Crohn's colitis + vitamin E + $KBrO_3$ and CS-Crohn's colitis + sulfasalazine. Rats were fasted for 24hours; thereafter, Crohn's colitis was induced by intra-rectal administration of NaOH. Colon weight and ulcer scores, Colon, liver and bones biochemical and histological analyses were also evaluated on days 3 and 7 post induction. Data were analysed using descriptive statistics, and significant at $p \leq 0$ .05.

Colon weight, weight/length, ulcer score was significantly decreased in $KBrO_3$ group compared with CU. Liver malondialdehyde content was significantly decreased in $KBrO_3$ treated group compared with CU group, while the liver protein, gluthathione, and nitric oxide level were significantly increased. A significant increase in the myeloid:erythroid ratio of the bone marrow in the (CVK) $KBrO_3$ and vitamin E treated groups compared to the untreated group. However, the histopathological analysis shows relative preservation of the colon mucosa in the CK and CVK groups both at days 3 and 7 compared with CU group. More so, there was normal sinusoid, hepatocyte morphology in the $KBrO_3$ treated groups when compared with the control.

Potassium bromate attenuated the adverse effect of increased liver oxidative stress, and reduced myeloid erythroid ratio in the bone marrow thereby enhancing immune response during crohn's colitis healing in male Wistar rats.

Key words:

Colitis, potassium bromate, hepatoprotection, osteohomeostasis, oxidative stress.

AGE-RELATED CHANGES IN PARIETAL CELL TURN-OVER AND APOPTOTIC CELL CLEARANCE DURING HEALING OF ACETIC-ACID-INDUCED GASTRIC ULCER

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Ageing is associated with functional and structural mucosal defense defects and delayed gastric ulcer healing. Gastric parietal cells play an important role in gastric mucosal homeostasis as well as coordination of physiological repair.However, paucity of research exist on how gastric parietal cell turn over may affect gastric ulcer healing in different ages, hence this study.

Forty (40; n=10) male Wistar rats were divided into 4 groups based on their ages as 3-, 6-, 12- and 18-months old. Gastric

ulcer was induced using acetic acid (0.2mL of 30%) and the ulcerated stomachs were harvested after 3 and 7 days for macroscopic ulcer scoring, mucin content, histology and immunohistochemical expression of proliferating cell nuclei antigen (PCNA). Parietal and apoptotic cells were evaluated in the histology and used to compute percentage parietal cell turn-over and apoptotic cell clearance. The data were analysed using ANOVA at $p \leq 0.05$. The results showed decreasing gastric ulcer healing rate with ageing. There was a significant age-related decrease in gastric mucin content, percentage parietal cell turn over and percentage apoptotic cell clearance in the gastric ulcer margin. This correlated with decrease expression of PCNA in the ulcerated gastric mucosa with ageing. The delay in gastric ulcer healing with advancing age may be due to decreasing capacity of parietal cell turnover and apoptotic cell clearance with advancing age considering their significance in ulcer repair processes and cell proliferation.

Key words: Ageing, Ulcer healing, Parietal cell turn over, Apoptotic cell clearance, Proliferating Cell Nuclei Antigen (PCNA).

EFFECT OF MONOSODIUM GLUTAMATE (MSG) ON RENAL OXIDATIVE STRESS, TOXICITY AND HAEMATOLOGICAL PARAMETERS IN BREASTFEEDING WISTAR RATS

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This study assessed kidney toxicity and alterations in haematological indices caused by monosodium glutamate (MSG). Twenty (20) breastfeeding Wistar rats were separated into four (4) groups of five animals each (n=5); the MSG groups received varying doses of MSG at 925 mg/kg, 1850 mg/kg, and 3700 mg/kg. The normal control group received 1 ml/kg of distilled water. Administration lasted for fourteen (14) days; all doses were given orally. At the end of the administration, all animals were sacrificed and blood was collected for haematological and some biochemical assessments. Kidney tissues were excised and homogenized for the assessment of renal oxidative stress biomarkers. Malondialdehyde (MDA) concentration increased significantly ($p < 0.05$) in all the MSG groups compared to the control. Antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) decreased significantly ($p < 0.05$) in MSG groups compared to control. There was also a significant ($p < 0.05$) decrease in the level of reduced glutathione (GSH) in all the MSG groups compared to the control. Serum creatinine, total and conjugated bilirubin were significantly higher ($p < 0.05$) in all the MSG groups compared to the control. Red blood cells, White blood cells, mean corpuscular haemoglobin, mid-range absolute values, haemoglobin concentration and platelets were significantly reduced ($p < 0.05$) in the MSG groups compared to the control. However, mean corpuscular volume and granulocytes were significantly ($p < 0.05$) higher in the MSG groups compared to the control. In conclusion, oral administration of MSG at higher doses is detrimental to renal function and haematological indices during lactation.

Keywords:
Monosodium Glutamate, Renal, Toxicity, Haematological and Breastfeeding

EVALUATION OF RESPIRATORY SYMPTOMS AND WORK-RELATED FACTORS IN GRAIN MILLERS: CASE STUDY OF SABON-GARI, KADUNA.

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Grain millers are at high risk of developing respiratory symptoms due to contact with flour dust in their work environment. The study assessed respiratory symptoms and work-related factors among grain millers in Sabon-Gari Local Government, Kaduna. A descriptive cross-sectional study was conducted among 132 adult Grainmillers that were exposed to dust particles during milling process for at least one year. Grain millers with 1-10years of working experience constituted 40.2% of the participants, 45.6% of the grain millers worked for 7-12 hours per day. Up to 66.1% of the grain millers had cough, 25.2% wheezing, 50.4%shortness of breath, 25.2% chest tightness. Up to 30.7% of the millers had chronic chest problem, while 31.5% had chronic breathing problem. Grain millers that operated for up to13-18 hours per day had the highest incidence of cough. There was a significant association between the operating hours and incidence of cough, chest tightness, chronic chest problems and chronic breathing problems. Grain millers with 1-10years of working experience had the highest incidence of cough and wheezing. A significant association between years of working experience and incidence of cough was observed. There was significant association between years of working experience and chronic chest problems and chronic breathing problems. The prevalence of respiratory symptoms among grain millers in the study area was high.