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The Cephalometry of the Yoruba Ethnic Group of Southwestern Nigeria

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Summary: Cephalometry of an ethnic population is determined by sex, diet, geographic location and genetics. Quantitative facial morphometry is necessary in today's contemporary society because of the globalization of crime and justice. The objective of this study is to determine Yoruba ethnic population's cephalofacial uniqueness for gender identification. A total of 222 adults (155 females and 67 males) participants from 10 local government areas in 5 states of the South-west Nigeria were randomly selected. Pre-defined set of cephalometric parameters were measured using standard requirement for anthropometry. Statistical analysis was calculated for gender differences using SPSS 20. Overall, gender differences (male vs female) was exhibited in head length, head width, upper facial height, lower facial height and facial width. Sexual differences were also exhibited in head modulus index (41.43±1.72 cm Vs 42.87±2.18 cm) and the index of the size of head (2361.89±444.53 cm_3 vs 2147.78±316.13 cm_3). Both genders exhibited dolichocephalic/mesocephalic type. Gender identification in this ethnic group may concentrate on five facial morphometry.

Keywords: Cephalometry, Yoruba, Nigeria

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INTRODUCTION

Cephalometry is an aspect of biological anthropology that deals with measuring the head and face of living individuals for the assessment of a population's cephalofacial features. These characteristics in an individual, ethnic group and population results from the interplay of factors such as sex, diet, geographic location and genetic constitution (Argyropoulos and Sassouni, 1989; Bhatia *et al.*, 1955; Del Sol, 2005). The early research in human physical anthropology was focused on characteristic differences of the anthropometric and craniometric measurements between human races (Hall *et al*., 2005). A study by Guha (1935) revealed that anthropometric and craniometric measurements could be used to categorize individuals drawn from a range of ethnic populations. It has been shown that the human face shows variability in size and shape that confers individual and group uniqueness (Ersan, 2014). Identification of facial feature points is an important factor in video surveillance, face detection, face recognition, facial expression classification (Sohail and Bhattacharya, 2008). Ethnic populations require standards for comparison. Careful documentation of anthropologic differences and similarities allows one to distinguish heterogeneity and also provide the basis for the application of techniques in forensic science (Fix, 1979). Craniofacial anthropometry is important in forensic medicine, plastic and reconstructive surgery, orthodontics and clinical diagnosis of dysmorphism (Durtschi et al., 2009; Farkas, 1994). Previous studies have reported differences in craniofacial anatomy among racial groups and these have been documented in a variety of structures but the oral and maxillofacial regions have been shown to be a particularly of defining region of variability between different racial and ethnic groups (Enlow *et al*., 2005; Farkas *et al*., 2005; Mayo *et al*; 1999; Porter *et al*., 2004; Teck *et al*., 2000; Waters, 2000; Yokota, 2005). Comparative anthropometric analysis remains an important investigative tool for understand ethnic groups in countries with such social, cultural, and ethnic diversity as Nigeria. The maxillofacial size and shape differences are essential for determination of the sex and the accuracy of prediction can be up to 91.1% (Bejdova *et al*., 2018).

The hypothesis is cephalofacial characteristics can predict sex among Yoruba ethnic group. This study is to reveal Yoruba ethnic population's cephalofacial characteristics and its possible application in differentiation of gender.

MATERIALS AND METHODS

Sample population and ethical considerations

A total of 222 (155 females and 67 males) participants who are volunteers aged 18 years and above were recruited from 10 local government areas in 5 states of the South-west Nigeria. Sampling

fraction was based on Yoruba ethnic population (Yp) of southwest Nigeria (27,722,452) and Nigeria population (Np) of 140,431,790 (National Population Commission, 2010). The sampling fraction Yp/Np was 0.2, resulting in the selection of two volunteers out of every eligible ten Yoruba volunteers. The target sample size was to attain the minimum of 60-90 volunteers from the ethnic population (Bashalkhanov *et al*, 2009). Volunteers' inclusion criteria are 18 and above years of age; verified pedigree pattern for each volunteer to ensure that parents, grandparents and great grandparents descended from Yoruba ethnic group. Exclusion criteria include previous head injury with cephalofacial deformation, previous facial surgery, and congenital cephalofacial abnormalities.

The procedures outlined in the International Organization for Standardization (ISO) general requirements for establishing anthropometric data bases were followed (ISO15535, 2012).

The Ethical approval for the study was obtained from Ministry of Health Research Ethical Review Committee (Ethical approval number AD 13/479/620).

Cephalometric measurements

Cephalometric measurements were taken from voluntary participants according to internationally accepted landmarks for human anthropometry (Hall *et al.* 2007). All the measurements were taken by the same person to avoid inter-observer error. All parameters taken were recorded in a spread sheet from the field and transferred into a log book in the laboratory. Cephalometric variables (in centimetres) were taken on the right side of the volunteers using digital calliper (Mitutoyo, Illinois, U.S) calibrated to 0.01mm. Informed consent was obtained from each of the volunteers.

The measured parameters were as follows:

- Head circumference (HC),
- Head length (HL),
- Maximum biparietal diameter (MBD)
- Head (Skull) height (HH),
- Nasal height (NH),
- Facial height (FH),
- Upper facial height (UFH),
- Lower facial height (LFH,
- Bizygomatic distance (BZD) (facial width) (Hall *et al.,* 2007).

Calculation of cephalometric indices and Cranial/Cephalometry Index classification

The cephalometric indices were calculated according to Garson (1885) as follows:

$$\text{Cephalic Index (CI)} = \frac{\text{Maximum Head Breadth}}{\text{Maximum Head Length}} \text{ X } 100$$

$$\text{Head Lenght Index (HLI)} = \frac{\text{Head Height}}{\text{Maximum Head Length}} x\ 100$$

Schmidt's Head Modulus Index (SMHI) =
Max. Head Lenght + Max. Head Breadth + Head Height

Index of size of the Head (ISH) =
Max. Head Length X Max. Head Breadth X Max. Head Height

$$\text{Morphological Facial Index (MFI)} = \frac{\text{Facial Height}}{\text{Bizygomatic Breadth}} x\ 100$$

$$\text{Sagittal Naso-Facial Index (SNFI)} = \frac{\text{Nasal Height}}{\text{Morphological Facial Height}} x\ 100$$

Statistical analysis

Data are presented as Mean ± SD. Software package for statistical analysis (SPSS 20) was used to calculate the mean, standard deviation and T- test for gender differences within the population. Frequency distribution of head and facial morphology were estimated based on Linear Measurements.

RESULTS

In this study, the mean HC was 56.59±3.40 cm for both sexes. The female HC of 56.62±3.01 cm was not significantly different from male HC of 56.52±4.22 cm. The mean HL was19.43±1.03 cm for both sexes. The male HL of 19.95±1.17 cm was significantly higher than female HL of 19.21±0.87 cm ($p<0.05$).

Table I: Cephalometric parameters according to sex in Yoruba ethnic group

Variable	All (N=222)	Female (n=155)	Male (n=67)
Age (years)	49.90±17.94	47.78±18.33	54.97±15.97
Head circumference (cm)	56.59±3.40	56.62±3.01	56.52±4.22
Head length (cm)	19.43±1.03	19.21±0.87	19.95±1.17*
maximum biparietal diameter (cm)	14.69±0.88	14.58±0.78	15.08±0.99*
Head height (cm)	7.73±1.06	7.68±0.94	7.85±1.31
Nasal height (cm)	5.75±0.92	5.68±0.96	5.90±0.78
Facial height (cm)	12.52±1.45	12.40±1.30	12.81±1.72
Upper facial height (cm)	5.37±1.16	5.39±0.98	5.40±1.35*
Lower facial height (cm)	7.11±0.85	6.98±0.80	7.41±0.91*
Bizygomatic distance (cm)	13.30±0.97	13.10±0.89	13.76±0.99*

Mean ± SD *P < 0.05 Male versus Female

Table 2. Cephalometric gender indices of male and female Yoruba ethnic group

Variable	All (N=222)	Female (n=155)	Male (n=67)
Cephalic index (%)	75.69±4.25	75.67±3.87	75.72±5.05
Vertical index (%)	52.78±7.68	53.02±6.97	52.23±9.15
Height length index (%)	39.89±5.85	40.07±5.29	39.46±7.01
Morphological facial index (%)	94.36±10.86	94.83±9.93	93.24±12.78
Sagittal Naso-facial index (%)	46.02±6.82	45.63±4.81	46.92±10.04
Head modulus index (cm)	41.67±3.48	41.43±1.72	42.87±2.18*
Index of the size of head (cm^3)	2202.00±399.56	2147.78±316.13	2361.89±444.53*

Mean ± SD *P < 0.05 Male versus Female

Table 3: Percentage distribution of head and facial morphology in Yoruba ethnic population based on linear measurements

Head/Facial Type	Male		Female				
	Range	Frequency %	Range	Frequency %	chi-square	df	p value
VERY SHORT	≤16.90	0	≤16.1	0			
SHORT	17.00 – 17.70	1.6	16.20 – 16.90	15.8			
MEDIUM	17.80 – 18.50	19.5	17.00 – 17.60	63.2	1313	158	<0.0001
LONG	18.60 – 19.30	76.4	17.70 – 18.40	20.4			
VERY LONG	≥19.40	2.5	≥18.50	0.6			
	Maximum biparietal diameter (Head width)						
	Range	%	Range	%	chi-square	df	p value
VERY NARROW	≤13.90	6.1	≤134	7.1			
NARROW	14.00 – 14.70	11.8	13.50 – 14.10	39.2			
MEDIUM	14.80 – 15.50	36. 4	14.20 – 14.90	28.5	966.4	152	<0.0001
BROAD	15.60 – 16.30	39.6	15.00 – 15.70	21.4			
VERY BROAD	≥16.40	6.1	≥15.8	3.8			
	Bizygomatic diameter (facial width)						
	Range	%	Range	%	chi-square	df	p value
VERY NARROW	≤12.70	15.2	≤12.00	14.7			
NARROW	12.80 – 13.50	10.3	12.10 – 12.70	24.7			
MEDIUM	13.60 – 14.30	19.7	12.80 – 13.50	40.4	862.8	148	<0.0001
BROAD1	4.40 – 15.10	51.8	13.60 – 14.20	11.2			
VERY BROAD	≥15.20	3	≥14.30	9			
	Facial height						
	Range	%	Range	%	chi-square	df	p value
VERY LOW	≤11.10	2.1	≤10.20	3.8			
LOW	11.20 – 11.70	6.1	10.30 – 10.70	4.5			
MEDIUM	11.80 – 12.30	13.7	10.80 – 11.30	40.7	834.3	148	<0.0001
HIGH	12.40 – 12.90	33.6	11.40 – 11.90	35			
VERY HIGH	≥13.00	44.5	≥12.00	16			

+ Head and facial morphology Range according to Lebzelter and Saller classification (Singh and Bhasin, 2004).

The mean MBD was 14.69±0.88 cm in both sexes, the value in male of 15.08±0.99 cm was significantly higher than that of female of 14.58±0.78 cm. The HH, NH and FH were not significantly different in both sexes. UFH for both sexes was 5.37±1.16 cm, the male UFH of 5.40±1.35 cm was significantly higher than female UFH of 5.39±0.98 cm ($p<0.05$). The LFH in both sexes was 7.11±0.85 cm, LFH value in female of 6.98±0.80 cm was significantly lower than the male value of 7.41±0.91 cm ($p<0.05$). The BZD in both sexes was 13.30±0.97 cm, the female BZD was 13.10±0.89 cm, the male BZD of 13.76±0.99 cm was significantly higher than that of female (Table 1). Five of the cephalometric measurements revealed significant gender difference ($P<0.05$). The Yoruba male had longer, broader head and wider face than Yoruba female. The lower face was significantly longer in the male.

Seven cephalometric indices were calculated and compared between sexes of the Yoruba ethnic group. The CI for both sexes was 75.69±4.25%, CI showed no significant difference in male and female. The VI was 52.78±7.68% for both sexes, VI showed no significant difference for the male and female Yoruba ethnic group. The HLI, MFI, and SNFI indices were not significantly different in both sexes ($p>0.05$). The HMI for both sexes was 41.67±3.48 cm, the Yoruba male had HMI of 41.43±1.72 cm and female HMI was 42.87±2.18 cm. Male HMI was significantly higher than female ($p<0.05$). The ISH in both sexes was 2202.00±399.56 cm^3, male ISH of 2361.89±444.53cm^3 was significantly higher than that of female ISH of 2147.78±316.13 cm^3 ($p<0.05$). These indices also showed that the Yoruba male had higher dimensions of vertical height, length and breadth than the female and that male head had higher volume than the female head (Table 2).

Cephalometric range variations within the Yoruba male and female ethnic group was classified according to Lebzelter and Saller of head and face morphology (Singh and Bhasin, 2004) (Table 3).

The head morphology based on head length classification showed that 1.6% of male and 15.8% of female had short head, 19.5% male and 63.2% female had medium head, 76.4% male and 20.4% female had long head while 2.5% male and 0.6% female had very long head.

Head morphology based on maximum biparietal diameter (head width) showed that 6.1% of male and 7.1% female of the population had very narrow head width. 11.8% male and 39.2% female had narrow head width, 36.4% male and 28.5% female had Medium head width. 39.6% male and 21.4% female had broad head width while 6.1% of the male and 3.8% of the female had very broad head width.

Head morphology based on Bizygomatic diameter (facial width) revealed 15.2% of the male and 14.7% of the population had very narrow facial width. 10.3% male and 24.7% female had narrow facial width. 19.7% male and 40.4% female had medium facial width. 51.8% male and 11.2% female had broad facial width. 3.0% male and 9.0% female had very broad facial width.

Head morphology based on facial height showed that 2.1% male and 3.8% female had very low facial height, 6.1% male and 4.5% female had low facial height. 13.7% male and 40.7% female had medium facial height. 33.6% male and 35.0% female had high facial height while 44.5% male and 16.0% female had very high facial height.

DISCUSSION

The HC of the Yoruba female of 56.62±3.01 cm and male of 56.52±4.22 cm are similar with the results of cephalic anthropometry of the Igbo ethnic group (Esomonu and Badamasi, 2012) and of Oladipo *et al*., (2010) for Ijaw ethnic group. Fulani ethnic group of northern Nigeria HC as reported by Maina *et al*. (2012) was less than the HC of southern ethnic groups. Head circumference is an indicator of health and global cranial growth in early childhood (Gonzalez Bejarano *et al*., 2014). Multicentre longitudinal cohort study will be necessary to evaluate the effect of geographical location and ethnic diet on head circumference of Nigerian ethnic populations as well as establishing growth pattern by age, ethnic group and sex.

The HL, MBD, HH, NH, FH, UFH, FH, BZD are comparable to cephalofacial parameters from other Nigeria ethnic groups (Oladipo and Olotu, 2006; Oladipo and Paul, 2009), however, this study showed that linear measurements of head length, head width, facial width and lower facial height were less in values in females than in males. This may be due to the males being generally larger than females. Garson (1885) reported that craniometric measurements showed average of 5-9% larger measurements in males than females. Facial anthropometric measurements were also found to be of higher numerical values in the male than in the female in west African ethnic groups (Darko *et al*., 2017). The cranial measurements in determination of population affinity in South Africans also revealed larger measurements in males than females (Iscan and Steyn, 1999). Thai population also expressed larger cranial measurements in males than females (Mahakkanukrauh et al., 2015). Craniometric analysis of the modern Cretan population also showed that males are statistically significantly greater than females in all dimensions. Cephalometry is important for gender recognition and identification and had been reported that apart from pelvis, skull exhibits higher sexual dimorphism in human body (Janson *et al*., 2011; Kranioti *et al*., 2008; Fortes de Oliveira *et al*., 2012). The cephalometrics of HL, MBD, UFH, FH, and BZD in this study showed significant difference between male and female Yoruba ethnic population of southwestern Nigeria.

The cephalic index of 75.72% for Yoruba female and 75.67% for Yoruba male in this study classified the head type to be upper end of dolichocephalic and lower end mesocephalic according to Saller's Length Breadth index of head scale (Singh and Bhasin, 2004). Dolichocephalic and mesocephalic head type had been reported for the Yoruba ethnic population living other regions of Nigeria (Oladipo *et al*., 2015; Umar *et al*., 2011). Beals (1972) observed range of mesocephalics to be the characteristic head type for populations living in zones having a wet and hot climate. Change in CI was only reported for generation of migrants born under the new environmental conditions (Kobyliansky, 1983).

The head modulus index (HMI), and the Index of the size of head (ISH) showed sexual dimorphism within the Yoruba ethnic population. These indices are factors of head length and head width. The significant volumetric differences of female and male Yoruba ethnic group were due to cephalic length and breadth and not the height. The extent of growth of the head had been shown to have significant substantial involvement of genetic factors. The determination of head-size and head-shape by genetic traits has been firmly established (Jelenkovic *et al*., 2008; Jelenkovic *et al*., 2010; Karmakar *et al*., 2007). The skull is considered by Anthropologists to be the best indicator of ancestry as well as indicator of sex second only to pelvis in sex determination (Sanger *et al*., 2013). In medicolegal cases, identification of sex is of prime importance, skull had been found to be useful in this regard because of resistance to adverse environmental conditions over time (Sudke and diwan Chhaya, 2013). Heritability of specific facial traits had been shown to range from 28 to 67%, and that over half of facial traits of greater than 90% can be explained by common genetic variation (Cole *et al*., 2017). The value of the

facial measurements of the present study was subjected to frequency of head and facial types between Yoruba ethnic group sexes. 95.9 percent of male had medium/long head while 79 percent of female had short/medium head length. 76 percent of male had medium/broad head width while 67.7 percent of the female had narrow/medium head width. Male face are broader (51.8%) than the face of female (11.2%) in comparison to narrow/medium facial width of 65.1% of the female that had narrow/medium facial width. The higher percentage of male exhibited high/very high facial height (78.1%) while female had 75.7 percent medium/high facial height. These findings are in conformity with established anatomical principle that females have smaller crania with shorter facial features than males (Moore *et al*., 2006). The human face reveals differences between the sexes and this result indicates that different degrees of masculinity and femininity can be constructed from witnesses' description and/or facial morphometry which can be of use in forensic investigation. Thus, based on this study, all cephalometric values cannot distinguish male from female. The gender identity of the Yoruba ethnic group may rely on head length, head width, facial height, facial width and lower facial height.

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Age Related Histology and Immunohistochemistry of Some Intermediate Filaments in the Testis of the African Catfish (*Clarias Gariepinus*)

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Summary:
The African Catfish (*Clarias gariepinus*) are important source of protein for local consumers in developing countries in Africa and have also been reported to have enormous commercial potential. Several works have been done on plethora of general histological, biochemical and hormone changes which accompany puberty in African Catfish. Other studies have touched the effects of ecotoxins on the histological and reproductive parameters of the mature African Catfish. This study is an attempt to use immunohistochemical and basic histology to elucidate the baseline information on the general structural differences between the testes of immature and post-pubertal catfish with respect to some intermediate filaments arrangement within the testicular tissue. Ten (10) each of mature male catfish (4-5 months old) and immature male catfish (3 months old) were used in the study. The fish were subjected to cold shock and decapitated before the testes were harvested from both groups. These tissues were fixed in Bouin's fluid for 24 hours and subsequently transferred into 70% Ethanol. Testicular tissues from both groups were processed for paraffin embedding for routine staining with H&E; another set of tissues were fixed in 10% Neutral Buffered Formalin for testicular immunostaining techniques against Vimentin, Desmin, Cytokeratin and Smooth Muscle Actin (SMA) expression using standard methods. There is an increase in seminiferous luminal area/diameter in the mature catfish testis with the presence of mature spermatozoa in the lumen when compared with immature catfish testis which has small size of lumen with absence of mature spermatozoa. Testicular interstitium thickness remain relatively unchanged. SMA was markedly expressed in the cytoplasm of interstitial Leydig cells in the immature catfish testis whereas it was weak in its expression in the mature catfish. However, SMA was not expressed in the connective tissue proper in the testicular interstitium. Cytokeratin expression was also marked in the testicular capsule of immature catfish but was weak to absent in the mature catfish, however, both mature and immature catfish had moderate cytokeratin expression in their seminiferous tubule basement membrane. Desmin was strongly expressed in cytoplasm of immature germinal cells in the immature catfish testis but was moderate in its expression in the mature catfish testis. Vimentin expression was marked in the cytoplasm of immature germinal cells in both immature and mature catfish testis but weak in its expression in the Sertoli cell cytoplasm of both groups. This study infers that ultra-structural and protein changes can be related to age changes alone apart from the contribution of seasonality and external interference by ecotoxins. The age-related changes seen in this study could set ``baseline information. The extent of contribution of season and other external factors will be better understood. Though the age-related difference might be peculiar to the species of current interest, the differences elucidated are a sound background for relational studies, especially on the effect of ecologic toxins on immature testis, as separate from the mature testis.

Keywords*: Clarias gariepinus*; Testis; Vimentin; Desmin; Cytokeratin; Smooth Muscle Actin

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INTRODUCTION

The African Catfish (*Clarias gariepinus*) are important source of protein for local consumers in developing countries in Africa and also have been reported to have enormous commercial potential. Apart from these, they are currently being used in various biological researches centring on ecological and nutritive events that affect this species. Anatomical data on the structural framework of both developmental and mature features of the fish is very important.

The testis of the African Catfish are paired elongated lobes located within, in the dorsocaudal compartment of the celomic cavity. They are suspended by mesenteries (mesorchia) which attach them to the gas bladder. A thin tunica albuginea, forms the capsule of the testicular lobes. Each lobe is composed of a collecting duct (ductus deferens) aligned with the long axis of the lobes. Seminiferous

tubules radiate from sections off the entire length of the ductus deferens.

Spermatic tissue of fish histologically is composed of a tubular compartment which is made up of clusters of large germ cells. These are separated by an interstitial compartment of connective tissue (Lofts and Holmes,1985; Cristiane *et al.*, 2012). Intermediate filaments are relatively a lot more abundant than other structural proteins like microfilaments and microtubules in the skeletal frame of epidermal cells (Lodish *et al.*, 2000). Compared with microfilaments and microtubules, they have a greater stability, a larger assemblage of proteins and also exist in nature as dynamic polymers.

They are structural proteins which laminates the cellular and nuclear membranes, where they associate and interact with other cytoskeletal structures and receptors (Lodish *et al.*, 2000; Alberts *et al.*, 2002). They are most probable to be identified intact in cells even after different types of tissue processing (Lodish *et al.*, 2000; Alberts *et al.*, 2002).Several works has been done on the plethora of general histological, biochemical and hormone changes which accompany puberty in African catfish (Lofts and. Holmes, 1985; Cavaco *et al.*,1999; Nóbrega and Quagio-Grassiotto, 2007). Other studies have also touched the effect of ecotoxins on the histological and reproductive parameters of the mature African catfish (Sayed *et al.*., 2011; Sayed *et al.*., 2012). This study is an attempt to use immunohistochemistry and basic histology to elucidate the baseline information on the general structural differences between the testes of immature and post pubertal catfish with respect to some intermediate filament arrangements within the tissue.

MATERIALS AND METHODS

Ten matured male fish (4-5 months old) and ten immature (3 months old) male fish were used. The fish were subjected to cold shock and decapitated before harvest of the testes

Testicular tissues obtained from both groups and further divided into two portions. The first portion was fixed in Bouin's fluid for 24 hours before being transferred to 70% ethanol. The fixed tissue samples were processed, using routine paraffin embedding technique. 5µm thick testicular sections were prepared and mounted on specimen slides. These were stained with Harris' Hematoxylin and Eosin stain (H&E). The second set of testicular tissues was directly fixed in 10% Buffered Formalin, before the routine paraffin embedding for the immunostaining technique.

LSAB-plus kit (Dakocytomation, Denmark) was used for the immunostaining technique, performed on 5 µm-thick testicular sections. Deparafinization of the sections was done using xylene and standard grades of ethanol concentrations. The slides were immersed in hydrogen peroxide solution in water 3% (v/v) for 5 minutes to block endogenous peroxidase activity. The slides were then rinsed in a 0.01M phosphate buffer saline (PBS), solution 1-1 (pH 7.4) for 5 minutes. Thereafter, the slides were immersed in citrate buffer solution and microwaved at low heat(750 W) for 15 minutes .

After cooling, the sections were rinsed with PBS and incubated for 30 minutes at room temperature with standard dilutions of monoclonal antibodies against vimentin (1:100), desmin (1:300), cytokeratin (1:100) and smooth muscle actin(1:50). The slides were subsequently rinsed with PBS and then incubated for 15 minutes with a ready-to-use biotinylated secondary antibody (LSAB-plus kit, Dakocytomation, Denmark). Thereafter, the slides were rinsed in PBS and subsequently incubated for 15 minutes with the streptavidin component of the LSAB-plus staining kit. Slides were then rinsed in PBS and bound antibody was visualized after the addition of a 3,3' - diaminobenzidine tetrachloride solution (LSAB-plus kit, Dakocytomation, Denmark).

In the negative controls normal mouse serum was used as the primary antibodies. Intestinal tunica mucosa was used as a positive control for both desmin and smooth muscle actin, tissue from the salivary gland was used for cytokeratin whilst tissurom the tonsils was used as a positive control for vimentin.
On examination of the stained slides with a digital photomicroscope (VJ-2005 DN), the relative intensities of vimentin, desmin, cytokeratin and actin immunostaining were designated as absent (-), weak (+), moderate (++) and strong (+++) as described by Madekurozwa and Kimaro (2006).

RESULTS

There is an apparent increase in seminiferous luminal area/diameter in the matured testis which also showed presence of mature spermatozoa in the lumen, as seen in Figure 1.

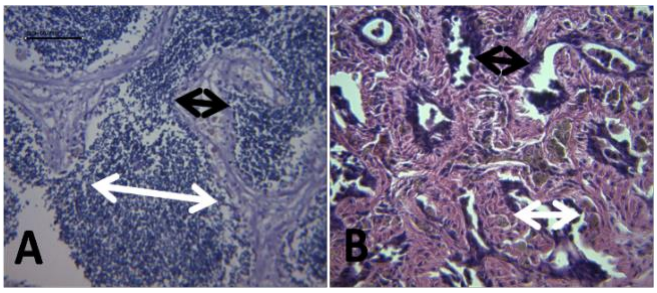

Figure 1
Light microscopy of Hematoxylin and Eosin (H&E) stained *Clarias gariepinus* testis (A) cross-section of the testis of the mature (A) and immature (B). Testicular organization with seminiferous lobes and interstitial tissue. Diameter across seminiferous lobes (white double headed arrows), interstitial thickness (black double headed arrows) MAG: X 400

While the thickness of the interstitium does not appear to increase relative to prepubertal to post pubertal age, the prominence in the immature is accentuated by the small size of the lumen and the absence of matured spermatozoa.

As shown in figures 2-5, Cytokeratin expression is found in the inner recesses of the testicular capsule and the basement membrane underlying the sertoli cells. The immature catfish testis has a more intense expression at these sites relative to the mature. Desmin is intensely expressed in the cytoplasm of immature germinal cell cytoplasm.

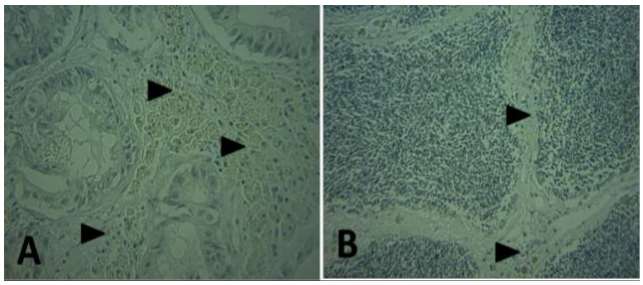

Figure 2:
Localisation of actin filaments in cells at the intertubular connective tissue (arrows) of groups A : Immature Testis ; B: Mature Testis.

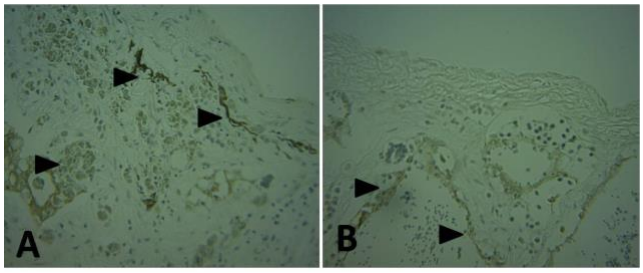

Figure 3:
Localisation of Cytokeratin filaments in cells at the testicular capsule and basement membrane of (arrows) of groups A : Immature Testis ; B: Mature Testis. MAG: X 400

The comparison of the intensities is summarised in Table 1. Actin immunostaining was well expressed in the cytoplasm of cells found in the testicular interstitium (Leydig cells). They are however not seen in the connective tissue that forms the interstitium proper. This expression is relatively higher in the immature testis. The Actin immunostaining is also present but poorly expressed in the sertoli cell cysts and immature germinal cell cytoplasm.

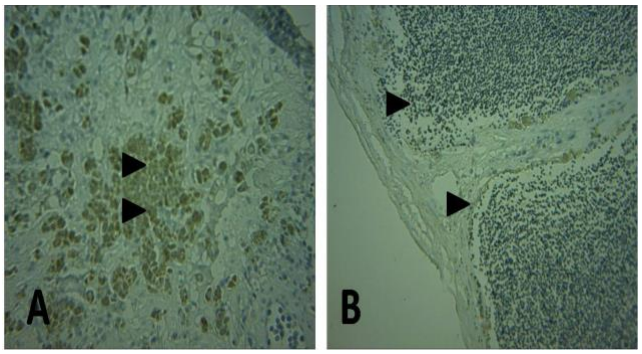

Figure 4:
Localisation of Desmin filaments in Immature germinal cells at the seminferous tubular lumina (arrows) of groups A : Immature Testis ; B: Mature Testis. MAG: X 400

These cells are relatively more abundant in the immature testis; the immune-expression appears more prominent though at the same intensity with the same site in mature testicular tissue. Vimentin expression is limited to the cytoplasm of immature germinal cells and they are also found lightly at the margins of sertoli cysts. The strength of expression appears the same in the two age groups of testicular tissues.

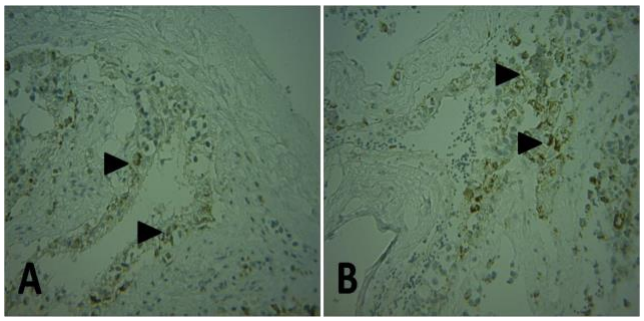

Figure 5:
Localisation of Vimentin filaments in Immature germinal cells at the seminferous tubular lumina and also at the margins of the sertoli cell cysts. (arrows) of groups A : Immature Testis ; B: Mature Testis. MAG: X 400

Table 1:
Summary of the immunohistochemical localization of the intermediate filaments; cytokeratin, vimentin, desmin and the microfilament-actin in the Mature and immature testis of the African Catfish.

Cell/Tissue stained	Actin		Cytokeratin		Desmin		Vimentin	
	A	B	A	B	A	B	A	B
Testicular Capsule	-	-	+++	+/-	-	-	-	-
Testicular interstitium	-	-	-	-	-	-	-	-
Basement membrane			++	++	-	-	-	-
Immature Germinal cell cytoplasm	++	+	-	-	+++	++	+++	+++
Sertoli Cell Cysts	+	+	++	+	-	-	+	+
Leydig cells	++++	+	-	-	-	-	-	-

Intensities of immunostaining: --, absent; +, weak; ++, moderate; +++, strong. A= Immatured, B= Matured.

DISCUSSION

Weak actin immune-expression was seen in interstitial cells, Sertoli cysts and germinal cells similar to what obtains in the study of cytoskeletal proteins in Mosquito fish (Arenas *et al..,* 1995). However, there is poor expression of actin immunostaining in the Sertoli cell cysts and immature germinal cell cytoplasm relative to the Leydig and contractile cells within the interlobular septum supports the assertion that intralobular and interlobular cell population are not homologous (Grier *et al..*, 1989).

There was no immunoreaction to desmin and very mild immunoreaction vimentin observed in the Sertoli cells of both immature and mature testes in this study. This is quite similar to the result of desmin and vimentin assay in Mosquito fish (Arenas *et al..*, 1995). Cytokeratin immunoreaction was clearly seen in the testicular capsule, germinal basement membrane and Sertoli cysts of both mature and immature African catfish, though the reactivity was more pronounced in the immature group.

The results of this study clearly indicate that the intermediate filaments in the testis of prepubertal and post pubertal testis exhibit disproportionate epithelial and non-epithelial tissue distribution pattern. Increase in germinal cell activity with respect to maturity in addition to other cellular and molecular components might be responsible for the enhanced seminiferous luminal area/diameter in the matured testis .The thickness of the interstitium at maturity does not appear to increase relative to pre pubertal age. The prominence of the interstitial connective tissue seen in the immature is accentuated by the small size of the tubular lumen, absence of matured spermatozoa and possible a smaller mass of the testis.

Biochemical and hormone changes have already been proven to accompany puberty in African catfish (Lofts and. Holmes, 1985; Cavaco *et al.,*1999; Nóbrega and Quagio-Grassiotto, 2007). Also, several works have indicated age, reproductive season and treatment related difference in quantity and distribution pattern of some proteins in the testis of African catfish (Raghuveer and Senthilkumaran, 2009; Raghuveer and Senthilkumaran, 2010; Rajakumar *et al..*, 2012). The relationship between age and protein distribution could be related with the effects of ecological factors on age and seasonality.

This study infers that ultra-structural and protein changes can also be related to age changes alone apart from the contribution of seasonality and external interference by ecotoxins. The age related changes seen in this study could set baseline information. The extent of contribution of season and other external factors will be better understood. Though the age related differences might be peculiar to the species of current interest, the differences elucidated are a sound background for relational studies, especially on the effect of ecologic toxins on immature testis, as separate from the mature ones.

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Age-Related Effects of Lead Poisoning on Some Haematological Parameters in Adult Wistar Rats

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Summary: The World Health Organization (WHO) estimates that, about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution, and that most of these environment-related diseases are however, not easily detected and may be acquired during childhood and manifested later in adulthood. The aim of this work was to evaluate sub-chronic effects of lead poisoning on haematological parameters and some sex hormones, as well as age-related changes on Wistar Rats. Thirty (30) of 3-, 5-, and 7-months old male Wistar rats, were divided into experimental (lead fed) and control (distil water) groups. Haematological parameters were determined, while blood lead concentration was determined using the method of Atomic Absorption Spectrophotometer. There was a significant ($P< 0.05$) increase (46.00, 46.75, 50.75 vs 14.56, 18.00, 17.60) in blood lead concentration with insignificant ($P> 0.05$) increase in the concentration of WBC counts (12.433, 13.000, 12.250 Vs 12.400, 10.000, 11.250) between the experimental and control groups. Significant decrease in Body Weight (77.43, 107.88, 134.35Vs 130.66, 150.60, 165.62), RBC counts (5.333, 7.000, 6.250 Vs 7.000, 7.500, 7.250), PCV (22.667, 40.00, 35.25 Vs 37.600, 45.5,43.25), Hb (10.000, 12.000, 10.75 Vs 13.200, 13.250, 12.50), MCV (45.333, 54.500, 55.750 Vs 55.400, 59.500, 58.250), MCH levels (15.00016.25016.500 Vs 18.400, 17.750, 17.000), as well as insignificant decrease in platelet counts(410, 373, 341 Vs 437, 313, 384), and MCHC (29.67, 29.75, 30.00 Vs 32.800, 30.25, 29.250). The effect of lead (Pb) on these parameters was observed to be more pronounced in younger animals ($P\leq0.05$). It was concluded that, ingestion of lead acetate produces more physiological derangement in young Wistar Rats.

Keywords*:* Hematological, Lead acetate, Wistar Rats.

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INTRODUCTION

Lead is a soft, malleable, and heavy metal with a bluish-white color which tarnishes to a dull grayish on exposure to air (Olade, 1987). Over the last three decades, there has been increasing global concern over the public health impacts attributed to direct and indirect environmental lead pollution, in particular, the global burden of disease. The World Health Organization (WHO) estimates that, about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (WHO, 2000). Most of these environment-related diseases are however, not easily detected and may be acquired during childhood and manifested later in adulthood (CCNM, 2014). The health of 200 million people in low-income countries is at risk from toxins such as lead or mercury, more than from AIDS, tuberculosis and malaria combined (C-Dynamics, 2014); nearly a quarter of deaths in developing countries, including Nigeria and Ghana are linked to pollution (MSN News, 2013).

Sources of occupational exposure to lead include mining, refining, smelting, construction work, paint removal, demolition, maintenance of bridges and water towers, car repair, ammunition, batteries, solder, X-ray shields and recycling (Pizent, et al. 2012).

Lead may be found in dirt, dust, house hold utensils, dishes, furniture, leaded petrol, paints, ceramics, food cans, make-ups, traditional remedies, batteries, soil and water of varying degrees of concentration; and lead poisoning usually occurs from repeated exposure to small amounts (Agency For Toxic Substances and Disease Registry (ATSDR) 1999). Lead has become a regulatory concern and subject of much interest because of its widespread distribution in environment due to its continuous emission from industrial sources, automobile exhaust and its pharmacological behaviour to remain bound to mammalian tissues for a long duration (Freeman, 1970).

Countries in the sub region of Africa, with the exception of the Republic of South Africa, have not implemented lead reduction programmes, and lead pollution has continued to pose health hazards in animal and man in Nigeria and many other parts of the world (Ajayi, Adeniyi and Babayemi, 2009).Leaded petrol, as one of the metal's sources in the environment, its usage levels is a good indicator of environmental lead exposure (Landrigan , et al., 2000).

There are many reports on lead toxicity and its deleterious effects in various species of animals as well studies on its pharmacokinetics and genotoxicity, but very few researchers tried to correlate haemato-biochemical alterations of lead acetate in laboratory animals especially in rats (Suradkar, *et al*, 2009). There are many reports on the effects of lead poisoning on various body systems, organs and blood parameters with limited studies on age- related effects of the heavy metal in relation to some hematological variables.

The purpose of the study was to evaluate the age-related sub-chronic effects of lead poisoning on haematological parameters in Wistar Rats.

MATERIALS AND METHODS

Materials used:

- Accubind Elisa Microwells, Monobind Inc Lake Forest; CA 92630. USA Product Code: 625-300) to estimate LH concentration in blood samples
- Accubind Elisa Microwells, Monobind Inc Lake Forest, CA 92630. USA; Product Code : 425-300) to estimate FSH concentration in blood samples
- Accubind Elisa Microwells, Monobind Inc Lake Forest, CA 92630. USA; Product Code: 3725-300) to estimate Testosterone concentration in the blood samples
- Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA) to estimate blood lead concentration in blood samples.

Methodology

Thirty (30) Wistar Rats of different ages of 3-, 5-, and 7-months old were divided into two groups of experimental and control groups respectively. The experimental (n=15), animals were fed orally with aqueous lead acetate solution at 250mg/kg body weight per day (Ambali *et al*., 2011) for 22 days, while the control (n=15) received distilled water.

Both control and experimental animals were acclimatized for 7 days prior to commencement of experiment; animals were housed in metallic cages and given free access to laboratory chow and water. After intervention, rats were anesthetized by intravenous injection of 0.5cm3 of 0.4% solution of sodium thiopental (Greene, 2002) and afterwards decapitated. Blood samples were collected through a glass funnel into two test-tubes for each rat: first sample collected in heparinised test-tubes was used for RBC, WBC and platelet counts as well as determination of PCV, MCHC, MCV values, and Hb concentration; Second sample collected in EDTA test-tubes was used for spectrophotometric analysis of blood lead concentration in the blood samples, using Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA).

Statistical Analysis

Data was collected and analyzed using student independent T-test to compare difference between experimental and control groups, while ANOVA was used to compare significant difference between experimental animals between the three age groups. All analysis were performed using Statistical Package for Social Science (SPSS) (Windows Evaluation Version 20, LEAD Technologies, USA) at p-value ≤ 0.05.

RESULTS

Effect of lead poisoning on weight gain

The initial and final body weights of the animals are shown table 1 while table 2 shows the weight(g) changes in the animals after experiment in different age groups of animals. Significant difference ($p \leq 0.05$) was recorded between experimental and control animals among all age groups. Significant changes in weights were recorded in all age groups, weight gain of 34.6g in normal control, while experimental had mean weight loss of 29.66g. Also, significant ($p \leq 0.05$) weights changes in 5-months old animals were also found with weight gain in normal controls (42.5g). However, a loss in weight was recorded in experimental group (28.25g). Mean weight gain of 16.25g was observed in normal control of 7-month old animals, though a loss was recorded in experimental animals (16.00g). There was no significant ($P > 0.05$) difference in weight loss between the three age groups.

Table 1:
Weight of Animals Before Experiment

Ages of Animals (months)	Weight(g) Group	
	Experimental	Control
3-month old	107.20±13.70	96.66±28.80
5-month old	136.20±17.99	110.98±18.62
7-month old	150.37±14.07	149.45±18.27

Table 2:
Weight Changes in Animals After Experiment

Ages of Animals (month)	Weight(g) Group	
	Experimental	Control
3-month old	77.43±3.38*a	130.66±28.80
5-month old	107.88±12.80*a	150.60±6.65
7-month old	134.35±8.97*a	165.62±17.80

Data with similar letter are not significantly ($P > 0.05$) different *$P < 0.05$ compared to control animals

Table 3:
Blood Lead Levels of Animals After Experiment

Ages of Animals (months)	Lead Levels(µg/dl) Group	
	Experimental	Control
3-month old	46.00±6.00*a	14.56±7.65
5-month old	46.75±18.95*a	18.00±3.65
7-month old	50.75±12.65*a	17.60± 4.50

Data with similar letter are not significantly ($P > 0.05$) different*$P < 0.05$ compared to control animals

Effect of exposure on the plasma lead level

Table 3 represents values of Blood lead concentration in different animal age groups. Significant differences ($P \leq 0.05$) were recorded in experimental compared to control groups. While no significant ($P > 0.05$) difference in Blood Lead levels between all age groups among experimental animals.

Effect of lead poisoning on haematological variables after exposure

The effect of lead exposure on the various measured haematological variables are shown Table 4.

Platelet counts: Platelet count was not significantly ($P > 0.05$) different between the experimental and control animals among all age groups. Similarly, no significant ($P > 0.05$) difference was observed between all age groups experimental animals.

Red blood cell (RBC): There was a significant ($P \leq 0.05$) reduction in RBCs was recorded in experimental animals of both 3- and 7-months old compared to control animals. While no significant ($P > 0.05$) difference in RBC Counts was observed between all age groups in the experimental animals.

White blood cell (WBC): There was no significant ($P > 0.05$) difference in the WBC between the experimental and control animals among all age groups.

Packed cell volume (PCV): Significant reduction ($p < 0.05$) was recorded between the experimental and control animals among all age groups. Also, the 3-months old animals had a significant ($P<0.05$) decrease in PCV levels compared to 5- and 7- months old experimental animals.

Haemoglobin concentration: Significant differences ($p \leq 0.05$) were recorded in experimental animals of 3- and 7-months old animals compared to their respective control groups. Also, a significant ($P < 0.05$) decrease in Heamoglobin levels between 3-months old compared to 5-months old experimental animals was recorded with more decrease among the 3-months old animals.

Mean Capsular Volume (MCV): A significant ($p \leq 0.05$) difference was recorded in experimental animals of 3- and 5-months old compared to control animals. While no significant ($P > 0.05$) difference in MCV between all age groups among experimental animals.

Mean Capsular Haemoglobin Concentration (MCHC): Significant ($p \leq 0.05$) reduction in MCHC was recorded in 3-months old experimental animals compared to the control. While no significant ($P >0.05$) difference in MCHC levels between all age groups among experimental animals.

Table 4.

Haematological parameters of the animals after the Experiment

Heamatological variables	Ages of Animals (months)	Group		P-Value
		Experimental	Control	
Platelet Counts(x10^3/µl)	3-month old	410±151.12 ^{a}	437±99.19	0.739
	5-month old	373±152.11 ^{a}	313±125.52	0.625
	7-month old	341±124.89 ^{a}	384±24.23	0.464
RBC Counts (x 10^6/µl)	3-month old	5.333±1.52*^{a}	7.000±0.70	0.027
	5-month old	7.000±0.58^a	7.500± 0.58	0.900
	7-month old	6.250±0.50*^{a}	7.250±0.50	0.035
WBC Counts (x 10^3/µl)	3-month old	12.433±2.30^a	12.400±0.55	0.944
	5-month old	13.000±0.82^a	10.000±4.08	0.313
	7-month old	12.250±5.19^a	11.250±1.89	0.757
PCV Levels (%)	3-month old	22.667±5.21*^{a}	37.600±5.46	0.024
	5-month old	40.00±1.41*^{b}	45.5±2.65	0.040
	7-month old	35.25±2.87*^{b}	43.25±0.96	0.045
Hb Concentration (g/dl)	3-month old	10.000±1.00*^{ac}	13.200±2.28	0.034
	5-month old	12.000±0.82^b	13.250±1.70	0.397
	7-month old	10.75±0.95*^{bc}	12.50±1.00	0.044
Mean Capsular Volume, MCV (fl)	3-month old	45.333±6.86*^{a}	55.400±4.22	0.019
	5-month old	54.500±1.00*^{a}	59.500±1.91	0.023
	7-month old	55.750±3.59^a	58.250±2.21	0.192
Mean Capsular Haemoglobin Concentration (MCHC) (g/dl)	3-month old	29.67±1.15*^{a}	32.800±0.84	0.001
	5-month old	29.75±0.96^a	30.25±2.87	0.709
	7-month old	30.00±1.41^a	29.250±2.21	0.510
Mean Capsular Haemoglobin, MCH (pg)	3-month old	15.000±2.65*^{a}	18.400±1.51	0.027
	5-month old	16.250±0.50*^{ac}	17.750±1.26	0.031
	7-month old	16.500±0.57^{bc}	17.000±2.00	0.571

*Data with similar letter are not significantly (P >0.05) different *P < 0.05 compared to control animals*

Mean Capsular Haemoglobin (MCH).

A significant ($p \leq 0.05$) reduction in MCH was recorded in 3- and 5-months old experimental animals as compared to control group. There was a significant ($P < 0.05$) decrease in MCH levels between 3-months old compared to 7-months old experimental animals was recorded with more decrease among 3-months old animals.

DISCUSSION

Result of this study showed that, sub-chronic lead poisoning led to significant loss of weight in all lead-treated animals. It was previously reported that, the action of lead in causing weight loss may be attributed to loss of appetite and gastrointestinal disturbances (Cezard and Haguenoer, 1992); as well as to interruption in absorption and overall metabolism of feed nutrients (Marchlewicz., *et al*, 2006). Even though there was no significant ($P > 0.05$) difference between the three age groups, lead's activity in weight loss was relatively more pronounced in young 3-months old animals.

Lead showed no significant ($P > 0.05$) effect on platelet count among experimental animals compared to the control groups and between age groups. However, on relative comparison, lead-treated groups showed lower platelet count compared to control groups; this is possibly due to platelet high susceptibility to oxidative stress (Mcmurry *et al*, 1995; Ohyashiki, Kobayashi, and Mashi, 1991). Other workers decumented a contrary view that lead intoxication causes a considerable increase in Platelet Count compared to the control (Saeed, 2015 and Suradkar, 2009), which may be due to thrombocytopenia (Sudakova *et al*., 1983) followed by thrombocytosis (Sudakova *et al*., 1983; Yagminas *et al*., 1990).

Among both 3- and 7-month age groups, lead showed significant decrease ($P \leq 0.05$) in RBC count, PCV and Haemoglobin Concentrations compared to control groups. This agrees with previous studies reporting effect of lead in reducing RBC count (Helmy *et al*., 2000; Klassen, 2001; Alexa *et al.*, 2002; Mugahi., *et al*, 2003; Noori *et al.*, 2003; Othman *et al.*, 2004; Teijon *et al*., 2006; Suradkar., *et al*, 2009; USEPA, 2009; Wahab, 2010; Toplan *et al.*, 2004; Hanan, and Riham, 2012; Ibrahim., *et al*, 2012; and Diefy, Sharkawy, Sayed, and Shehata, 2014).

Reduction in RBC counts is due to fact that, erythrocyte membrane is vulnerable to lipid peroxidation with limited capacity to repair its damaged components due to oxidative stress (Flora, Pande, Kannan and Mehta, 2004).

Such haematological alteration on erythrocyte are attributed to effect of lead on some erythrocyte enzymes (Calderon-Salinas *et al*., 1993) in addition to its effect on cell metabolism, interaction with some reactions where calcium is their secondary mediator and inhibition of some enzymatic activities like amino-levulinic acid dehydrase (ALAD) (Klassen, 2001).

Lead shortened life span of erythrocytes due to increased fragility of their cell membrane while the reduced haemoglobin production is due to decreased levels of enzymes involved in heme synthesis (Guidotti *et al.*, 2008). Nabil, (2012) reported elevation of plasma bilirubin level in lead exposure which is probably due to red blood cells destruction mediated by heme oxygenase.

Contrary to this, Golalipour, *et al*. (2007) reported increased RBC count in adult albino Wistar rats after lead acetate exposure, which they suggested to be due to reduced oxygen transfer and tissue hypoxia. They attributed that the bone marrow could overcome the lead toxicity because of subchronic exposure of low dose levels. No significant ($P > 0.05$) difference in RBC Counts between age groups in experimental animals was recorded.

Lead showed no significant effect on plasma WBC count in all experimental groups, nor between age groups. However, on comparison with controls, lead-treated animals showed higher WBC counts compared to control groups. Similarly, repeated lead exposure has been demonstrated to increase WBC count (Mcmurry., *et al*, 1995; Mugahi., *et al*, 2003; Okedran, 2010; Ibrahim., *et al*, 2012). This contradicts a report by Suradkaret., *et al*, 2009 that lead reduces WBC count.

Leukocytosis in lead acetate administered rats has been attributed to the lead-induced inflammation (Yagminas *et al.*, 1990) and direct toxic action of lead on leucopoiesis in lymphoid organs (Mugahi *et al.*, 2003).

While there was no significant difference in lead's action on WBC counts between age groups, there was a significant age-related decrease in PVC. It was also observed that, age-related effect of lead poisoning led to decrease in Hb concentration in 3-, 5- and 7-months old animals respectively. The effect of which, was found to be more pronounced in the lower-aged animals.

On contrary Ajayi, *et al*, (2009) reported that lead to significantly increase PCV, of which he possibly attributed to short duration of lead treatment (100mg/kg bwt intraperitonealy for 7days). This suggest an initial increase in PCV followed by a decrease, during lead exposure.

Lead decreases heme biosynthesis by inhibiting amino-levulinic acid dehydrase (ALAD) and Ferrochelatase activity (USEPA, 2009). In continuous exposure, lead affects heme biosynthesis through inhibition of cytoplasmic and mitochondrial enzymes (ATSDR, 1993). The presence of reduced PCV, RBC and Hb in lead-treated groups suggests that sub-chronic lead poisoning leads to the development of anaemia. The anaemia may be caused by reduced haemoglobin synthesis, haemolysis of mature and immature erythrocytes with reduced life span. Lead poisoning is

also being linked to the interference of hematopoietic progenitor development, copper metabolism and production of erythropoietin (Klauder and Petering, 1977; Osterode, Barnas and Geissle, 1991).

Lead also reduces MCV, MCHC and MCH (Golalipour, et al. 2007; Wahab, 2010; Diefy, Sharkawy, Sayed, and Shehata, 2014; Yagminas *et al*., 1990; Helmy *et al*., 2000; Teijon *et al*., 2006; Durgut *et al*. 2008; USEPA, 2009; Nabil *et al*. 2012 and Nuran, Gurer-orhan, and Nukhet, 2001. The effect of lead poisoning on MCH were found to be more in younger (3-months old) animals.

The presence of reduced MCV and MCHC in the present study indicates microcytic hypochromic anaemia in lead-treated rats; this agrees with earlier studies (Noori., *et al*, 2003, Suradkar., *et al*, 2009; Mugahi *et al*., 2007; Suradkar *et al*., 2009;Klassen, 2001; Reichelmayyr-Lais, and Kirchgessner, 1984). The decresed MCHC and MCH is attributed to efffect of lead on haemoglobin synthesis. But Ambali et al 2012; Sherif , 2014 reported increased MCV in lead exposure in their studies, and also reported macrocytic hypochromic anaemia in lead treated animals.

Lead increases WBC Counts and significantly reduces RBC related indices in the experimental group and these effects were more pronounced in younger age group.

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Alterations in Gonadal Oxidative Stress Markers and Reproductive Function of Balb/C Mice Infected with Plasmodium Berghei

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Abstract: Infertility is generally regarded as a major clinical problem, and it adversely affects people both psychologically and medically. In this study, the changes in gonadal oxidative stress markers and reproductive function of BALB/c mice were investigated. Forty-eight (48) BALB/c mice acquired for this study were randomly divided into four (4) groups of eight (8) mice each. Each group was further sub-divided into male and female groups with equal number of mice. The groups were represented as thus: Group A: normal mice; Group B: mice infected with *Plasmodium berghei*; Group C: *Plasmodium berghei* infected mice treated with Artemether/Lumefantrine; Group D: *Plasmodium berghei* infected mice treated with Vitamin E. The experimental mice were inoculated with the *Plasmodium berghei*, and the parasites were confirmed in the mice four days later before the commencement of the experiments. After the experimental procedures which lasted for fourteen (14) days, the mice were sacrificed, blood samples collected for serum testosterone, estrogen and progesterone assay; semen were collected for semen analysis; and testes and ovaries were harvested for histological analyses and oxidative stress marker determination. Result show that *Plasmodium berghei* significantly ($p<0.05$) decreased the sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology. The parasites also decreased the serum concentrations of testosterone and progesterone. *Plasmodium berghei*, also caused significant ($p<0.05$) reductions in testicular and ovarian activities of superoxide dismutase, glutathione and peroxidase catalase while significantly ($p<0.05$) increasing the malonaldehyde level. The parasites also caused marked histological distortions in the testes and ovaries of the mice. Treatment with Artemether/Lumefantrine and Vitamin E separately reversed the detrimental changes induced by the parasites by increasing the semen quality and hormonal concentrations. Treatment with Artemether/Lumefantrine and Vitamin E also decreased the oxidative stress level of the gonads and improved the histological features of the testes and ovaries of the infected mice. This study therefore showed *Plasmodium berghei* infection posed anti-fertility threat while treatment with Artemether/Lumefantrine and Vitamin E ameliorates the effect of the parasites.

Keywords*: Plasmodium berghei*, Semen Quality, Oxidative Stress, Infertility, Artemether/Lumefantrine, Vitamin E.

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INTRODUCTION

Infertility has been established as a psychological, clinical and social problem which has proved a difficult challenge for both couples and medical professionals. Worldwide, about 10 to 15% of couples would have encountered this problem in their lifetime (Kliesch, 2014). In Nigeria, estimation from the demographic health surveys (DHS) data revealed a prevalence rate of 11% while epidemiologic and clinic-based studies suggested rates of between 14.5% and 30% (Adegbola and Akindele, 2014).

Infertility is a disease affecting the reproductive system, and it is defined as the inability or failure to achieve pregnancy after a year of regular and unprotected intercourse (Purvis and Christiansen, 1992). The male reproductive system is highly sensitive to numerous drugs, chemicals and infections which have shown deteriorating potentials on reproductive capacity under certain circumstances (Bonde, 1996). Oxidative stress generated due to the activities of free radicals triggers a range of pathophysiological changes that influence the reproductive functions generally in women and men (Said et al., 2005). Also, reactive oxygen species (ROS) has been advocated to play a huge role in infertility especially in unexplained (idiopathic) infertility (Agarwal et al., 2003). Apart from reactive oxygen species (ROS), reactive nitrogen species (RNS) are also considered to play diverse and extensive roles in many of the physiological and pathological events (Akaike and Maeda, 2000). When there is an imbalance between the pro-oxidants and antioxidants, with increase in pro-oxidants, oxidative stress (OS) arises that leads to excessive molecular damage and thrn tissue injury (Januel et al., 2006).

Oxidative stress has already been associated with progression of many ailments which include atherosclerosis, cancer, neurodegenerative diseases, rheumatoid arthritis etc (Sohail et al., 2007; Aruoma, 1998). However during malaria infection, the role of oxidative stress is closely monitored and it still remains unclear and controversial on whether it has a protective role or related to tissue pathological diseases (Becker et al., 2004; Pabon et al., 2003). These free radicals are continuously generated during normal aerobic metabolism and their production are equally removed by a variety of exogenous and endogenous antioxidants (Gutteridge, 1995).

Malaria infection induces the generation of hydroxyl radical in different organs, which is accountable for the creation of oxidative stress and possible apoptosis (Guba et al., 2006). Nitric oxide (NO•) is another molecule that have been shown to have an important role in pathogenesis of malaria (Hunt and Stocker, 1990). The malaria parasite itself has also been reported to generate large amount superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Mishra et al., 1994).

Malaria is transmitted by female anopheles, that is, it is a mosquito-borne infectious disease, and caused by the Plasmodium species, which include *Plasmodium vivax, Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae* and *Plasmodium ovale.* In early 21st century, malaria was described as endemic in over 104 countries and about 3.4 billion people were at risk for contracting the infectious disease. Most of the malaria cases globally are caused by *Plasmodium vivax* and *Plasmodium falciparum* (WHO, 2013). *Plasmodium falciparum* infection is considered as life threatening and has been attributed to most of the malaria-related deaths when compared to *Plasmodium vivax* which is described as relatively benign (WHO, 2013). There are similarities in characteristic between *Plasmodium berghei* and *Plasmodium falciparum* (Sherman, 2003). Both species of Plasmodium cause pathological damages and apoptosis in liver leading to complications in liver and other systemic tissues (Sand et al., 2005; Kochar et al., 2003). Some of the induced pathological conditions include damage of vital organs such as lungs, liver, spleen etc, and possibly anaemia (Jense et al., 2006).

Since the late 20th Century, several end products of artemisinin have been synthesized and then studied. Artemisinin which is an extract of the plant *Artemisia annua* is a highly effective drug for the treatment of *P. falciparum* malaria (Adekunle et al., 2009). Artemether is one of the derivatives of Artemisinin and its tablets have also proved to be effective in treating malaria infection, these drugs have gradually replaced Chloroquine and also Quinine for the treatment of malaria (Li et al., 1994).

In recent years, the potential of dietary antioxidants to eliminate the hydrogen peroxide (H_2O_2) and superoxide (O_2^-) radicals generated during malaria infection has received increased attention (Hug et al., 2003; Murugavel and Pari, 2004). The dietary nutrients have shown promise protective capabilities in lipid-soluble antioxidants such as vitamins A and E, lycopene, α- and β-carotene in humans because of their association with membrane lipids (Matzger et al., 2001). Amongst the various Vitamins, Vitamin E (α-tocopherol) acts as the most potent lipid soluble antioxidant (Frei, 1991).

The rate of malaria infection in Sub-sahara Africa is on the increase, coupled with the rate of infertility (Ranson and Lissenden, 2016). Several studies have highlighted the adverse effects of malaria in reproduction. In past reports, controversies have been generated on the association between gonadal oxidative stress status and fertility. Some researchers have shown reductions in oxidative stress markers in gonads of infertile men (Sanocka et al., 1997; Alkan et al., 1997), many have not (Zini et al., 2000; Hsieh et al., 2002). Artemether Lumefantrine anti-fertility effect is yet to be established (Morakinyo et al., 2009), but has potency in clearing malaria parasite and reducing toxic activities of the parasites from tissues. Hence this study will also assess the ameliorative role of Artemether Lumefantrine and Vitamin E on the gonadotoxic effect of *Plasmodium berghei*.

MATERIALS AND METHODS

Drugs

Artemether/Lumefantrine in tablets (Mekophar Chemical Pharmaceutical Joint-stock Company, Vietnam) and Vitamin E (α-tocopherol) capsules (Strides-Colab Ltd., India) were both purchased from the Pharmacy of Irrua Specialist Teaching Hospital, Irrua. Artemether/Lumefantrine tablets grinded into powdered form with a glass mortar, was mixed with distilled water and administered as aqueous suspensions by oral gavage at 0.9 mL kg^{-1} b.wt according to procedure of previous studies (Morakinyo et al., 2009). The drug suspensions were continuously stirred during administration in order to avoid sedimentation and to deliver the drugs homogeneously to the animals. Vitamin E (α-tocopherol) was also given by oral gavage in its oily formulation at a dose of 0.2 mL kg^{-1} (Sheweita et al., 2001).

Animal Handling

The study protocols were reviewed by the ethics committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The *Plasmodium berghei* NK65 strain were obtained from the Biochemistry Division, Nigerian Institute of Medical Research (NIMR), Yaba, Nigeria. Forty-eight (48) BALB/c albino mice weighing 20g – 25g and 8 – 10 weeks old, used in this study were acquired and

maintained in facilities of the Animal Unit, Faculty of Basic Medical Sciences, Delta State University, Abraka. The mice were kept in fibre glass cages, with wood shavings as beddings and had access to water and food *ad libitum*

***Plasmodium berghei* Inoculation and Estimation**

The recipient mice were infected with *P. berghei* by the passage of the malaria parasite from the donor animals through intraperitoneal route as by the methods of David et al. (2004) and Peter and Anatoli (1998). Briefly, *P. berghei* infected red blood cells were first collected from the orbital vein of malaria infected mice and this was diluted with Phosphate Buffered Saline (PBS). Each of the recipient mice were injected with 0.2 mL of the diluted blood so that the infected mice contained approximately 10_6-10_7 infected red cells (parasite) per kilogram of body weight. Presence of malaria parasite was confirmed in the recipient mice after four (4) days of inoculation, this was done using thin smears of blood films made from orbital vein of mice (David et al., 2004). The thin smears were stained with 10% Giemsa at pH 7.2 for 15 min and examined under the microscope to assess level of parasitemia. After confirmation of the malaria parasite in the mice, the experiment commenced.

Study Design

The study was experimental in nature. The animals were randomly divided into four groups of twelve (12) mice, each group was further subdivided into two sub-groups of male and female mice (at proestrous stage of the estrous cycle), with equal number of animals. The respective groups were treated as follows; Group A (Control) received normal saline, Group B: mice infected with *Plasmodium berghei*, Group C: *Plasmodium berghei* infected mice treated with 56mgkg$_{-1}$bwt Artemether/Lumefantrine and Group D: *Plasmodium berghei* infected mice treated with 150mgkg$_{-1}$ bwt of vitamin E. The administration of the saline, Artemether/Lumefantrine and Vitamin E were done orally for fourteen (14) days. At the end of the experiments, the mice were sacrificed and samples collected for histological and biochemical analysis.

Determination of Estrous Cycle

A vaginal swab was collected from a restrained female mouse using a cotton tipped swab that was wetted with physiological saline and inserted into the vagina of the animal. This swab was gently rolled against the vaginal wall with minimal animal discomfort and then removed. The cells acquired from the vagina were transferred to a dry glass slid, this was done by rolling the swab across the slide. The slide dried and then stained with 400 µL of stain (Accustain, Sigma-Aldrich, St. Louis, MO). The stained slides were rinsed with water, then covered with a coverslip, and viewed immediately at magnification of x200 under bright illumination. Mice whose vaginal swab contained more of nucleated and few cornified epithelial cells, and leukocytes were confirmed to be at proestrus stage (Felicio et al, 1984). Only female mice at proestrus stage of estrous cycle were selected for this study.

Histological Study

Testis and Ovaries were harvested and immediately preserved by placing in 10% formalin solution and later transferred into Bouin's fluid for longer fixation period. After fixation, the tissues were placed into separate ascending grades of alcohol with the purpose of dehydration. After dehydration, the tissues were cleared in two different changes of xylene, and then finally embedded in paraffin wax. With a rotary microtome, specimens were sectioned at 5µm and sections were mounted on clear slides and stained with haematoxylin and eosin. The slides were air dried and examined under the microscope using a magnification of x100 objective.

Semen Analysis

Immediately after dissection of the mice's abdomen, the testes were harvested and then semen were collected via aspiration from the epididymis. The procedure involved making minor incisions in the caudal portion of right ductus deference of the testis. Drops of semen were placed on the microscope slide and drops of warm 2.9% sodium citrate for every drop of semen were added. This mixture was then covered with the cover slip and examined under the microscope using a magnification of x40 objective with reduced light. Sperm counts, percentage of sperm with normal morphology and percentage of sperm with progressive motility were carried out using the new improved Neubeur's haemocytometer counting chamber.

Serum Hormonal Analysis

Serum was obtained from blood sample collected into plain bottles and assayed for testosterone, estrogen and progesterone concentrations. The hormone, testosterone was measured by using DRG Diagnostics testosterone kit, Germany, while estrogen and progesterone concentrations were analyzed using Monobind CA kit, USA. The three hormones were analysed according to the protocols of the manufacturer's kit.

Analysis of Biochemical Parameters

Lipid-peroxidation was ascertained by measuring the Malonaldehyde activities (MDA) using the procedure of Varshney and Kale (1990) and the values were expressed as nanomolar (nmol) of malondialdehyde (MDA) per gramme tissue. The level of superoxide dismutase (SOD) activity was assessed using the method of Mistra and Fridovich, (1972). Catalase activity was also following the guidelines of Sinha (1972). Glutathione peroxidase activity (GPx) was

measured as prescribed by the method of Rotruck et al. (1973).

Statistical Analysis

Data generated in this study were presented as mean ± SEM for four mice per group. One-way Analysis of Variance (ANOVA) was used to compare means while a post hoc test (Least Significant Difference) was further carried out used to assess the statistical significance of the data. A value of p-less than 0.05 was considered to be statistically significant. IBM SPSS (version 20) software was used to analyse the data

RESULTS

Changes in Semen Quality due to *Plasmodium berghei* Infection

Plasmodium berghei adversely affected spermatogenic activities as significant ($p<0.05$) decrease in the mean sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology was observed in the infected mice depicted in Table 1. Artemether/Lumefantrine was observed to inhibit the anti-fertility effect of the *Plasmodium berghei*.

It was also observed that Artemether/Lumefantrine increased the percentage of sperm with progressive motility and percentage of sperm with normal morphology respectively. The increase in percentage of sperm with progressive motility was also significant ($p<0.05$) when compared with *Plasmodium berghei* malaria infected mice. Similarly improvement in semen quality was also observed in *Plasmodium berghei* infected mice treated with Vitamin E, with significance ($p<0.05$) in percentage of sperm with progressive motility and normal morphology when compared with the values of *Plasmodium berghei* infected mice.

Effect of *Plasmodium berghei* infection on serum levels of reproductive hormones

Table 2 shows that *Plasmodium berghei*, caused an adverse effect on testosterone secretion as a decrease in serum concentration of testosterone in male mice was observed when compared to control. Minimal decrease in serum estrogen level of the female mice was observed following infection from *Plasmodium berghei*. Data from Table II also showed that *Plasmodium berghei* caused a significant ($p<0.05$) reduction in the serum concentration of progesterone in the female mice. These decrease in serum testosterone and progesterone concentrations were countered with Artemether/Lumefantrine and Vitamin E treatment in the infected mice. Despite the ameliorative effects of Artemether/Lumefantrine and Vitamin E treatments, statistical significance were not recorded.

Alteration in the level of Oxidative stress markers of *Plasmodium berghei* infected mice.

Data from Table III show the changes in oxidative stress markers of testis and ovary in mice infected with *Plasmodium berghei*. It was observed that there was significant ($p<0.05$) increase in the oxidative stress level in the testes and ovaries of malaria infected mice. Data show that *Plasmodium berghei*, significantly ($p<0.05$) decreased the activities of superoxide dismutase, catalase and glutathione peroxidase, while increasing the malonaldehyde activities with significance ($p<0.05$) when compared with the oxidative stress markers in control's testes and ovaries. Further, treatment with Artemether/Lumefantrine and Vitamin E decreased the testicular and ovarian oxidative stress level. Artemether/Lumefantrine increased the gonadal oxidative stress markers of mice infected with *Plasmodium berghei* with no statistical significance. On the other hand, treatment with Vitamin E significantly ($p<0.05$) increased the superoxide dismutase and catalase activities of the testis. It also increased the ovarian superoxide dismutase and glutathione peroxidase level while decreasing the malonaldehyde activities with significance ($p<0.05$) when compared with oxidative stress markers of *Plasmodium berghei* infected mice.

Table 1:
Effect of Plasmodium berghei on Semen Quality

	Control	Pb infection	Pb + ACT	Pb + Vitamin E
Sperm Count (x10$_6$ cells/mm$_3$)	75.00 ± 5.00	49.17 ± 8.41	56.67 ± 6.15	60.00 ± 5.77
Sperm with progressive Motility (%)	65.83 ± 3.52	25.00 ± 3.65*	42.50 ± 3.10*$_+$	60.83 ± 2.71$_+$
Sperm with normal morphology (%)	77.50 ± 3.10	50.83 ± 7.79*	62.50 ± 4.79*	73.33 ± 2.47*

*: $p < 0.05$ compared with Control group; +: $p < 0.05$ compared with *Plasmodium berghei* infected group (n = 6)

Table 2:
Effect of *Plasmodium berghei* on male Testosterone and female Estrogen and Progesterone

	Control	Pb Infection	Pb + ACT	Pb + Vitamin E
Testosterone (g/dL)	1.02 ± 0.09	0.77 ± 0.09	0.87 ± 0.09	0.97 ± 0.15
Estrogen (pg/mL)	75.00 ± 5.00	49.17 ± 8.41	56.67 ± 6.15	60.00 ± 5.77
Progesterone (ng/mL)	1.05 ± 0.07	0.75 ± 0.13*	0.93 ± 0.05	0.92 ± 0.10

*: $p < 0.05$ compared with Control group (n = 6)

Effect of *Plasmodium berghei* infection on the Histology of Testis

Fig. IA showed normal features of the testis were observed in the control mouse testis, but infection by *Plasmodium berghei*, caused distortion of the seminiferous tubules and interstitial cells of Leydig (Fig. IB). This detrimental change caused arrest of spermatogenic development in the testis. In Fig. 1C, Artemether/ Lumefantrine reversed the detrimental effect of the parasite, with minimal spermatogenesis and mild interstitial degeneration. Vitamin E treatment caused an improvement in the cellular structures of the testis in *Plasmodium berghei* infected mice with defined seminiferous tubules and essentially normal Sertoli cells and interstitial cells of Leydig (Fig. ID).

Effect of *Plasmodium berghei* infection on the histology of ovary: Micrograph in Fig. II shows blood vessels, corpus luteum, maturing oocytes, granulosa cells and corona radiata, follicular antrum and granulosa cells in ovary of control female mouse. It was observed in Fig. IIB that *Plasmodium berghei* infection caused degenerated oocytes, also degenerating granulosa cells were also observed in the ovary of *Plasmodium berghei* infected mice. Artemether Lumefantrine reduced the effect of *Plasmodium berghei* infection as mild congestion observed in the ovary (Fig. IIC) compared with the severe derangement of the histo-architecture of ovary in *Plasmodium berghei* infected mice (Fig. IIB). These derangements in ovary induced by *Plasmodium berghei*, appeared to be healed or reversed by Vitamin E treatment (Fig. IID) as the histology of the ovary showed similar features with control.

Table 3:
Effect of Plasmodium berghei on gonadal oxidative stress level

	Gonad	Control	Pb infection	Pb + ACT	Pb + Vitamin E
SOD (U/mgHb)	**Testes**	74.30 ± 6.09	32.88 ± 5.62*ь	40.49 ± 5.68	57.63 ± 5.35
	Ovaries	63.63 ± 4.80	30.49 ± 6.88*ь	42.26 ± 9.15	56.98 ± 3.78
Catalase (U/mgHb)	**Testes**	21.62 ± 3.04	9.26 ± 1.38*ь	11.97 ± 2.45	16.41 ± 1.58
	Ovaries	18.52 ± 3.03	11.81 ± 2.01*	13.15 ± 1.69	15.27 ± 1.58
GPx (U/mgHb)	**Testes**	17.22 ± 2.21	9.07 ± 2.08*	13.74 ± 3.59	15.86 ± 2.19
	Ovaries	18.35 ± 0.79	7.40 ± 0.99*ь	13.18 ± 1.53	16.31 ± 0.41
MDA (nmol/gm tissue)	**Testes**	110.70 ± 5.45	178.76 ± 27.40*	147.75 ± 15.22	126.47 ± 22.53
	Ovaries	95.58 ± 9.82	168.35 ± 12.62*ь	142.11 ± 17.86	71.82 ± 13.74

*: $p < 0.05$ compared with Control group; ь: $p < 0.05$ compared with Vitamin E group (n = 6)

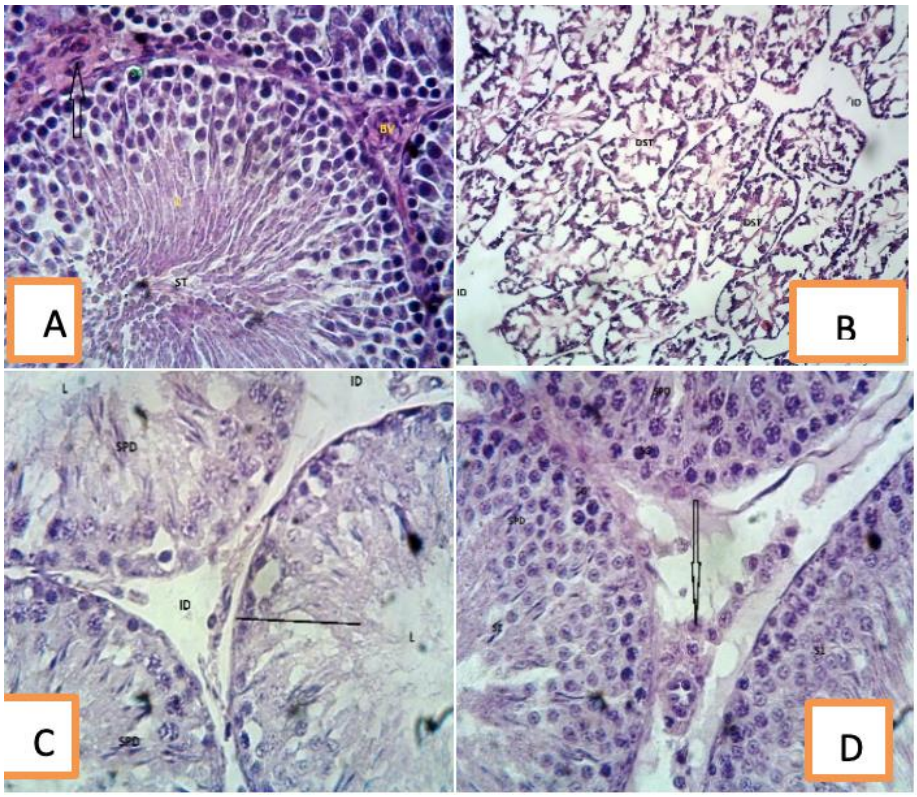

Figure 1:
Cross sections of histology of testis in control mouse (A), *Plasmodium berghei* infected mouse (B), *Plasmodium berghei* infected mouse treated with Artemether/Lumefantrine (C) and *Plasmodium berghei* infected mouse treated with Vitamin E. Stains: Haematoxylin & eosin, Magnification: x400.

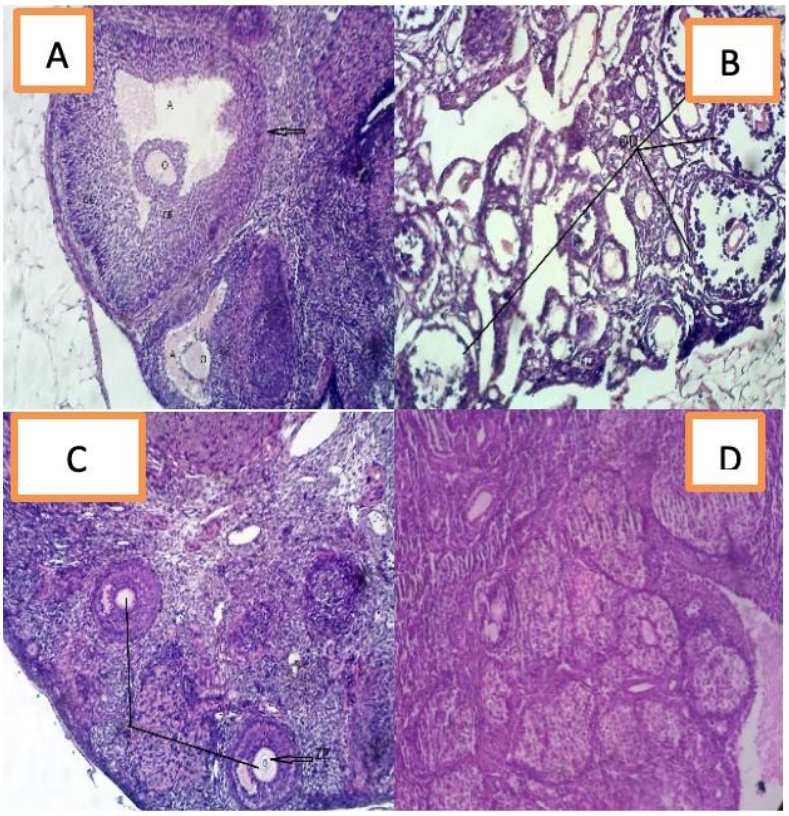

Figure 2:
Cross sections of histology of ovary in control mouse (A), *Plasmodium berghei* infected mouse (B), *Plasmodium berghei* infected mouse treated with Artemether/Lumefantrine (C) and *Plasmodium berghei* infected mouse treated with Vitamin E. Stains: Haematoxylin & eosin, Magnification: x100

DISCUSSION

Malaria is one of the leading cause of mortality and morbidity especially in developing countries, and this remains a serious public health issue in malaria endemic area of the World (Breman et al., 2004). Oxidative stress has been linked with pathogenesis of numerous disease conditions amongst which include malaria. The present study was carried out to examine the effect of *Plasmodium berghei* infection on male and female reproductive system (Agarwal et al., 2005).

In this present study, increase in oxidative stress level was established as *Plasmodium berghei* decreased the gonadal concentrations of superoxide dismutase, catalase and glutathione peroxidase, whilst increasing the malonaldehyde activities. The increase in MDA level is attributed to the insufficient antioxidant defence system that is manifested by decrease in the activities of testicular and ovarian catalas, superoxide dismutase and glutathione peroxidase. Findings from Kulkarni et al. (2003) reported that the decrease in levels of antioxidants during malaria infections and this is responsible for increase in oxidative stress. Conversely, Pabón et al. (2003) showed that elevated oxidative stress during malaria infection is also responsible for the increase in lipid peroxidation rather than from antioxidant decrease.

Plasmodium berghei, induced pathological changes in the testes and ovaries of the mice. In Fig. IB, degenerative changes caused by *Plasmodium berghei*, showed altered structural integrity of seminiferous tubules and interstitial cells of Leydig of the testis. This adverse changes is attributed to the increase in oxidative stress markers as recorded in Table III. This assertion was further confirmed by Sharma et al. (2006) whose study showed attenuating and toxic changes of *Plasmodium berghei* leading to disruption of antioxidant and pro-oxidant balance, by continuous generation of reactive oxygen species (ROS). In support to the findings of this study, Sibmooh et al. (2006) also showed that oxidative stress is a common phenomenon in acute malaria infection, hence the alteration in testicular histo-architecture observed in this present study, was induced by increased oxidative stress markers following infection from *Plasmodium berghei*.

Similarly, the membrane degeneration observed in testicular and ovarian tissue distortion is as a result of increased lipid peroxidation induced by *Plasmodium berghei,* this claim was established with increase in tissue malonaldehyde (MDA), an important lipid peroxidation marker (Cabrales et al., 2011). Apart from lipid peroxidation, *Plasmodium berghei* effect in decreasing other antioxidants such as catalase, gluathatione peroxide and superoxide dismutase in the testis and ovaries is another mechanism for gonadal histo-architectural damage. Evidence from reports of previous studies have shown that the activities of SOD and other antioxidants are decreased due to the infection in *Plasmodium berghei*-infected cells (Rodrigues and Gamboa. 2009; Farombi et al,. 2003). Studies from Sibmooh et al. (2000) and Das et al. (1993) have also previously shown that during malaria

infection, the presence of oxidative stress includes increased plasma lipid peroxidation, depletion of antioxidants and alteration of erythrocyte membrane flexibility.

This present study showed that *Plasmodium berghei* decreased the production of testosterone in male mice. This can be understandable with the testosterone synthesis function of interstitial cells of Leydig (Miller and Auchus, 2011) and due to the parasite inducing histological distortions in the interstitial cells of Leydig, hence impairing the testosterone producing function of the testis. The decreased serum progesterone level in female mice following *Plasmodium berghei* infection is also a reflection on damage on the ovarian integrity with increase in oxidative stress. Data generated from Reddy, Mahipal and Subhashini, (2006) showed that oxidative stress during *Plasmodium berghei* infections were related with momentary generation of pro-inflammatory mediators such as inducible nitric oxide synthase, interleukin 1β and cyclo-oxygenase-2. Also, Lalita et al., (2012) also showed the presence of apoptotic cells in tissues with increased oxidative stress and infected with *Plasmodium berghei*

Plasmodium berghei significantly ($p<0.05$) decreased the sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology. Testosterone is required in large local concentrations to maintain the process of spermatogenesis. Decrease in testosterone may hamper the production of sperm cells. Malaria parasites decreased the testosterone level in report from Muawia and Nabiela (2009), another possible reason for the decrease in sperm parameters due to *Plasmodium berghei* from this study. The distortion in testicular histo-architecture would result to decrease in sperm production (Orth, 1993).

Treatment with Artemether/Lumefantrine inhibited the damaging effects of *Plasmodium berghei* as the mean sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology in the malaria infected mice remained within control limits. This was in line with Akinlolu et al. (2007) that rats exposed to separate doses of Artemether Lumefantrine for seven (7) days showed normal morphological structures of the testis with evidence of spermatogenesis occuring. The semen quality also remained intact or within control range because of the normal level of testosterone in Plasmodium berghei infected mice treated with Artemether Lumefantrine. Similar findings from Morakinyo, Oludare, Ojulari et al. (2009), showing that serum testosterone level remained normal after administration of Artemether/Lumefantrine. Artemisinin-Combination Therapies ACT for which Artemether Lumefantrine is one, is known for its rapid parasite clearance (Price, Nosten, Luxemburger et al., 1996). This malaria parasite clearing property of Artemether Lumefantrine inhibited the increase in gonadal oxidative stress and hence limited the destructive influence of Plasmodium berghei on the testis and ovary.

Similarly, Vitamin E administration reversed the effect of malaria parasite in the mice by decreasing the oxidative stress level, improving the semen quality and increasing the serum concentration of the reproductive hormones. The reduction in oxidative stress level due to the effect of Vitamin E could play a huge role in the recovery of the fertility potential of the Plasmodium berghi infected mice. Akpotuzor et al. (2007) showed that antioxidant concentrations in malaria patients were lower than the levels for the control, suggesting that the reduction in antioxidant vitamins during malaria is attributed to increased function of the host's serum antioxidants caused by the malaria parasites to counteract oxidative injuries. This further confirms the beneficial role of Vitamin E in preventing the detrimental effect of *Plasmodium berghei* on reproductive function in this study.

The observation in this study confirms that *Plasmodium berghei* impairs gonadal function. The separate use of antioxidants supplements and anti-malaria drug, Artemether Lumefantrine were effective in ameliorating the anti-fertility of *Plasmodium berghei* through the decrease in gonadal oxidative stress level.

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Effects of Castration on Epidural Administration of Lidocaine-Tramadol in West African Dwarf (Wad) Goats.

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Summary: Epidural anesthesia is routinely used in ruminants for obstetric manipulations and caudal surgical procedures owing to complications associated with general anaesthesia in this species. Castration is a common farm practice for derived production benefits. The responses of a castrate to anaesthesia may differ from that of an intact animal because of possible anaesthetic-hormonal interplay. This study compared the anaesthetic indices, haemato- biochemical parameters (PCV, Hb, WBC, PLT, Na^{+}, K^{+}, Cl^{-}, urea, creatinine, Cu^{2+}) between castrated and intact goats subjected to epidural anaesthesia with tramadol-lidocaine mixture. Experimental animals were six West African Dwarf (WAD) goats (3 intact and 3 castrated bucks). The drugs were administered into the lumbosacral epidural space. Heart rate, respiratory rate and rectal temperature at 15minutes interval for 90minutes and anaesthetic indices were taken. Blood was obtained for haematology and serum chemistry before drug administration and hourly thereafter for three hours. The onset of drug action in the castrated goats (1.7±0.9 min) compared well with that of the non-castrated goats (2.0±0.0min). However, the duration of analgesia was significantly shorter ($p<0.05$) in the castrated goats (26.7±5.2min) than in the non-castrated goats (83.7±20.8 min), while the duration of recumbency was significantly ($p<0.05$) longer in the castrated goats (23.3 ± 8. 6min) than in the non- castrates (14.8 ± 3.7min). Mean heart rates ranged between 96.0±6.1 to 116.0±16.2 beats/min for non- castrated goats and 94.7±14.8 to 121.0 ±8.1beats/min for the castrated goats. Mean respiratory rates ranged between 60.0±14.4 to 89.3±16.2 breaths/min and 61.0± 31.5 to 122.3±10.0 in the non –castrated and castrated goats respectively. Mean temperature ranged between 39.6±0.4 to 40.8±0.4°C in the non-castrated goats and 40.3±0.3 to 41.4±0.3°C in the castrated goats. Anaesthetic indices and haematobiological parameters were compared using Student's t-test, while physiological parameters were compared using ANOVA for repeated measures. There was reduction in pre-treatment values of some of the haematological, biochemical and hormonal values especially urea in the non- castrate (pre-treatment value-of 22±4.16 and 3-hour post treatment value of 13.3±0.33). The combination of tramadol-lignocaine epidurally is safe in castrate animals as well as in intact animals especially West African dwarf goats as there were no deleterious effects. However, further studies could combine adrenaline for prolonged recumbency when required.

Keywords: Castrate, Epidural, Lidocaine, Tramadol

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INTRODUCTION

Castration, the removal of the testes, is a routine management practice in farm animals especially goats for described production benefits including prevention of inbreeding and removal of buck taint (Yami, 2008). A castrate differs from an intact goat in the testosterone level. The responses of a castrate to anaesthesia may differ from that of an intact animal because of possible anaesthetic-hormonal interplay. Indeed in humans, there have been some reports of decreases in testosterone level following general anaesthesia, possibly because of surgical trauma, but more often an increase occurred one hour after surgery (Cartensen *et al*., 1973).In another study to investigate the reliability of hormone concentrations in hormonal studies following anaesthesia, it was found out that up to 24 h after ketamine/xylazine anaesthesia, any measurements of plasma testosterone concentration, and after anaesthesia and CO2 euthanasia, LHRH concentration, should be treated with caution, as the real effect of the experiment or treatment could be hidden by the anaesthesia and euthanasia.

Ruminants are generally not considered good subjects for general anesthesia mainly because of the hazards of regurgitation and inhalation of ruminal contents or saliva into the lungs if the air way is left unprotected. Other complications associated with general anaesthesia in this species include ruminal tympany and marked cardiopulmonary depression (Taylor, 1999). As a result, regional anesthesia produced by perineural or epidural injections of anaesthetic agents is most frequently employed in this species (Hall *et al.*, 2001; Sadegh *et al.*, 2009). Lumbosacral epidural anesthesia is simple, inexpensive, the most common epidural technique and requires no sophisticated equipment. It is routinely used in ruminants for obstetric manipulations, caudal

surgical procedures and as an adjunct treatment for control of rectal tenesmus (Lee *et al.*, 2003; Sakrda and Tranquilli, 2007).

Tramadol is a synthetic opioid and has been administered via the epidural route in veterinary medicine (Halder and Bose, 2000; Guedes *et al.*, 2005). Two complementary models define its mechanism of action. The first results from the binding of its (+) enantiomer to mu-opioid receptors, whose affinity is about 6000 times less than that of morphine (Fantoni and Mastrocinque 2002; Bozkurt, 2005). However, its main active metabolite, O-desmethyltrama dol,is 6 times more potent than tramadol and possesses a 200-fold greater affinity for mu-opioid receptors (Pypendop and Ilkiw, 2007). The second mechanism involves the inhibition of noradrenaline reuptake by the (-) enantiomer through the increased release of serotonin and inhibition of its reuptake by the (+) enantiomer (Sousa *et al.*, 2007). Various studies suggest that tramadol produces a local and spinal anaesthetic effect while also increasing the postoperative analgesic period and reducing the consumption of analgesics (Delilkan and Vijayan, 1993; Kapral *et al.*, 1999).

Lignocaine is an acetamide local anaesthetic. Local anaesthetics act by blocking signal conduction by altering the fast voltage-gated sodium channels at the neuronal cell membrane (Lemke and Dawson, 2000). Because of its short duration of action, supplemental analgesia using different drugs or re-administration of drugs during surgical operations is usually necessary (Lemke and Dawson 2000; Skarda and Tranquilli, 2007). Opioids and alpha-2 adrenergic agonists are commonly used in combinations with lidocaine resulting in longer and adequate analgesia (Bigham*et al.*, 2009; Rostami and Vesal, 2012).

Tramadol is an analgesic with mixed opioid and non -opioid activities (Garrido et al. 2000) and has been administered into the epidural space alone or with lignocaine in humans (Batra et al., 1999), horses (Natalini and Robinson, 2000), dogs (Guedes*et al.*, 2005; Almeida *et al.*,
2010), cats (Castro *et al.*, 2009) lambs and goats (Dehkordi*et al*, 2012) with demonstration of prolonged analgesia and / antinociception. The mixture of tramadol with lignocaine was therefore chosen as a model in this study for preliminary investigation of possible peculiar effects of extradural anaesthesia on castrated goats in comparison with intact ones. Specifically, this study was to compare the physiological parameters, anaesthetic indices, and haemato- biochemical parameters between castrated and intact goats subjected to epidural anaesthesia with tramadol-lignocaine mixture.

MATERIALS AND METHODS

Animals

Six clinically healthy adult (3 intact and 3 castrated) WAD goats (bucks) were used for this study. The weight range of the goats was between 18 and 24kg with a mean± sem of 21±0.0kg. The goats which were all intact at purchase were obtained at an open market in Ibadan, Nigeria. They were housed together in a large pen that allowed them ample movement. They were fed with concentrates, maize, cassava peelings and unripe plantain. Water was provided ad libitum. The goats were dewormed with boluses of albendazole (Salbezole®, Sam Pharmaceuticals Ltd, Lagos) at an oral dosage of 5mg/kg body weight.

Drugs

Lignocaine hydrochloride (Glocain®, Vital Health Care PVT Ltd, India) which was supplied as 20mg per ml of colourless, aqueous solution without adrenaline in a 20-ml multidose vial. Tramadol hydrochloride (Tramaden®, Laborate pharmaceutical, India) supplied as 100mg in 2ml vial for injection

Experimental design

The goats were randomly allocated to two groups of three animals each. The group tagged the castrate were castrated six weeks before the commencement of the trials using burdizzo as previously described (Yami, 2008). Each animal in both groups was injected extradurally with lignocaine (2.46mg/kg) and tramadol (1mg/kg) mixed in the same syringe. The mean body weight of the castrates was 21.3 ±0.2kg while for the intact goats was 21.4±0.4 kg. The goat's rectal temperature (RT) and respiratory rate (RR) were immediately measured after the epidural injection and subsequently at 15 minutes intervals over a 90-minute period in the course of the trials. Respiratory rate in breaths/min was determined by visual observation of the thoraco- abdominal excursion. Heart rate was measured in beats/min with the aid of a precordial stethoscope. Rectal temperature was determined using a mercury-in-glass thermometer and measured in degrees centigrade (˚C). Venepuncture was done at the jugular vein to obtain blood before drug administration at 1, 2 and 3-hours post administration for haematology and serum biochemistry.

Experimental procedure

The goats were restrained manually on sternal recumbency with the hind limbs extended cranially. An area of 5-7cm was clipped generously and prepared surgically for sterile procedure. The lumbosacral junction was located as described by Hall *et al.*, (2014). In order to achieve a relatively painless epidural puncture, a skin bleb was made over the lumbosacral junction with 0.5ml lignocaine solution.

A 21g hypodermic needle was inserted at the lumbosacral junction (using the pelvic protuberance as a landmark to locate the depression) and then advanced into the epidural space. To confirm the presence of the needle in the epidural space, there was a lack of resistance to the injection of air and absence of spinal fluid in the needle cap. The syringe which contained a

calculated amount of local anaesthetic agent was attached to the needle and then injected over a period of time. The development of motor and sensory blockade was assessed by the goat's inability to stand on its hind limbs. Serial pricking of the skin of the goat's hind limb, perineum, flank and ventral abdomen caudal to the umbilicus with a needle (twenty-one gauge) as in previous studies (Dehkordi*et al*, 2012) was used to determine the onset and extent of analgesia on all or none basis.

Calculations

Onset of action: This was defined as the time interval in minutes between extradural drug injections to hind limb paralysis.

Duration of recumbency: This was defined as the time interval in minutes between onset of hind limb paralysis and ability to stand.

Duration of analgesia: This was defined as the time interval in minutes between time of loss of reflex response in hind limbs, perineum, flank and ventral abdomen to pricking with needle to return of sensation to those parts.

Haematology and serum biochemistry: Haematologic, biochemical and hormonal responses were evaluated from pre- treatment, 1, 2 and 3hours post treatment blood collections.

Statistical Analysis

All data was expressed as Means ± SEM. The mean indices of castrate and intact goats were compared using the student-t-test for paired data. The mean values of the measured physiological parameters were compared using the analysis of variance (ANOVA) for repeated measures followed by the Least Significant Difference (LSD) as post-test. A value of $p \leq 0.05$ was considered significant. Mean values of the haematologic, biochemical and hormonal responses of the castrate and non-castrate goats were compared using student T test.

RESULTS

Observations: Following the administration of extradural anaesthetic solution, neural blockade was consistently achieved in all the experimental goats. All the goats had hind limb paralysis except one castrate which was only mildly ataxic. However, there was a lot of thrashing about by the goats in an attempt to stand up while motor paralysis lasted.

Anaesthetic Indices: The anaesthetic indices of non-castrated and castrated WAD bucks are shown on Table 1. The onset of drug action in the castrated goats (1.7±0.9 min) compared well with that of the non-castrated goats (2.0±0.0 min). However, the duration of analgesia was significantly shorter ($p<0.05$) in the castrated goats (26.7±5.2min) than in the non-castrated goats (83.7±20.8 min), the duration of recumbency was significantly ($p<0.05$) longer in the castrated goats (23.3±8. 6min) than in the non-castrates (14.8±3.7min).

Physiological variables: The mean HR, RR and RT responses of the castrated goats and non- castrated goats after extradural tramadol-lignocaine anaesthesia are shown in Figures 1, 2 and 3 respectively. Mean heart rate ranges were 96.0±6.1 to 116.0±16.2 beats/min for non- castrated goats and 94.7±14.8 to 121.0 ±8.1beats/min for the castrated goats.

Table 1:

Anaesthetic indices of the extradural injection of lignocaine-tramadol in non-castrate and castrated WAD bucks.

	Non-castrate	Castrate
Onset of action (min)	2±0	1.7±0.9
Duration of analgesia (min)	83.7±20.8	26.7±5.2*
Duration of recumbency (min)	14.8±3.7	23.3±8. 6*

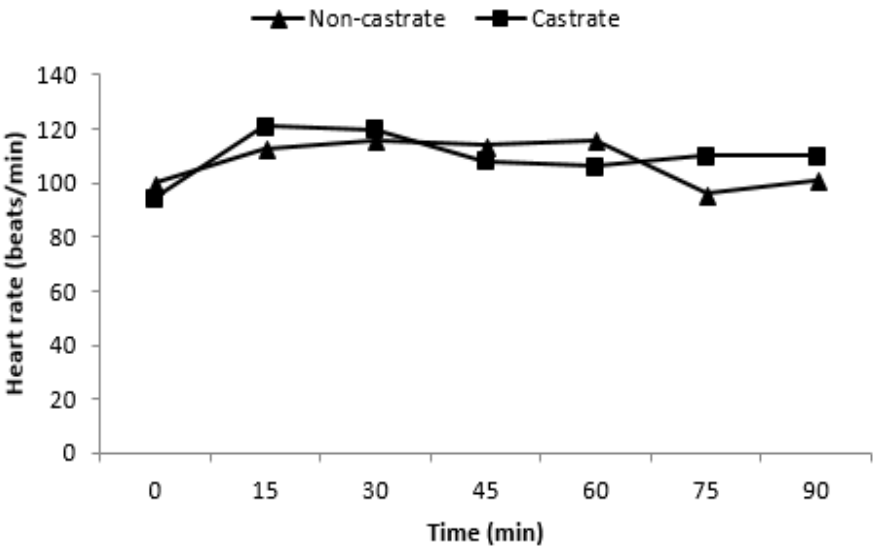

Figure 1.
Heart rate responses of non-castrated and castrated WAD bucks to extradural injection of lignocaine-tramadol mixture

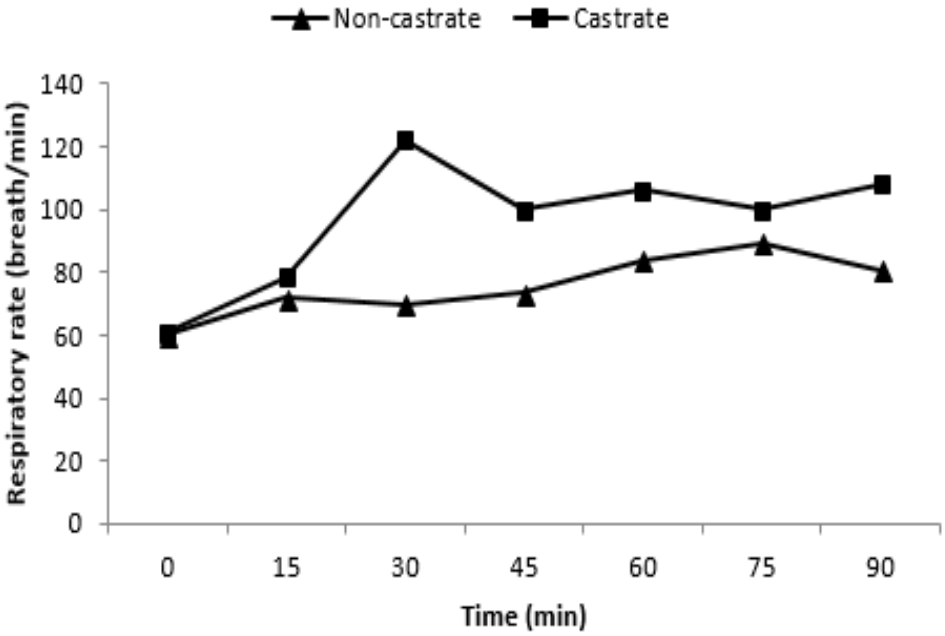

Figure 2.
Respiratory rate responses of non-castrated and castrated WAD bucks to extradural injection of lidocaine-tramadol mixture.

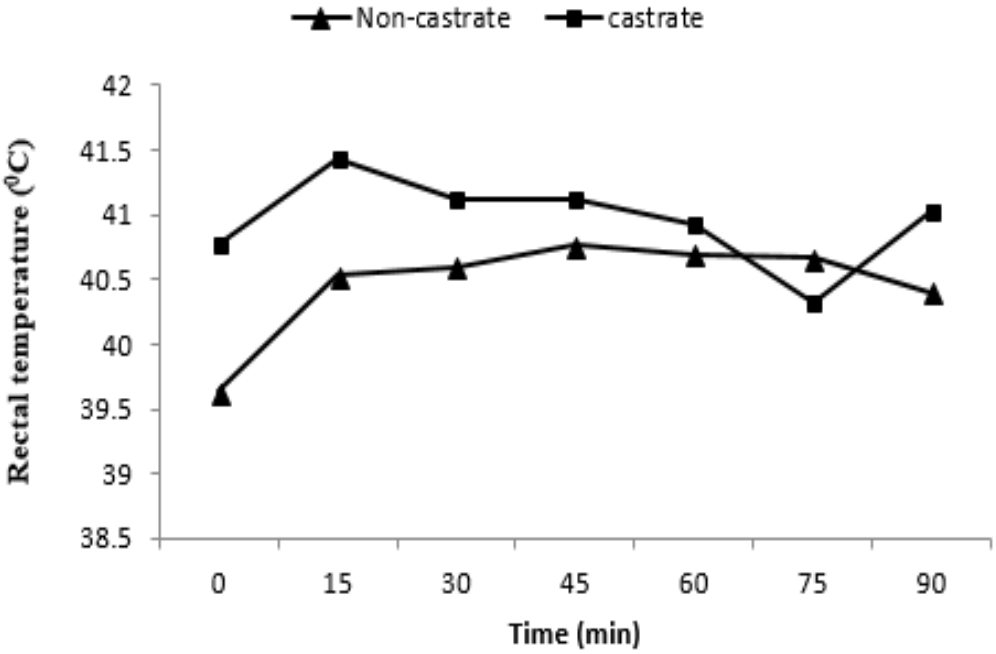

Figure 3
Rectal temperature responses of non-castrated and castrated WAD bucks to extradural injection of lignocaine-tramadol mixture.

Mean respiratory rate ranges were 60.0±14.4 to 89.3±16.2 breaths/min and 61.0± 31.5 to 122.3 in the non –castrated and castrated goats respectively. Mean temperature ranges were 39.6±0.4 to 40.8±0.4°C in the non-castrated goats and 40.3±0.3 to 41.4±0.3°C in the castrated goats.

***Haematology, biochemical and hormonal responses*:** There were flunctuations in the haematological parameters between the castrate and non-castrate goats pre and post treatment (Table 2). However, the castrates showed generally lower haematological values (PCV, Hb, RBC and WBC) than the castrates. The copper values were higher in castrates than the non castrates and showed significant differences ($P \leq 0.05$ and $P<0.01$) at 1 and 2 hours respectively post treatment. Urea values also showed significantly lower differences in non castrates than castrates post administration of tramadol-lignocaine (Table 2).

Table 2:
Haematology, Biochemical and Hormonal responses of non-castrated and castrated WAD bucks following lignocaine-tramadol extradural analgesia.

Parameters	Before Administration	Post-administration		
		1HOUR	2HOURS	3HOURS
PCV (%)				
Non-Castrate	39.33±0.88	33±0.58	39±1.15	30.33±0.67
Castrate	23±1.0	22.67±2.4*	24±1.15***	23±1.15**
Hb (gm/dl)				
Non-Castrate	13.67±0.33	12.3±0.7	15.17±0.6	12±0.57
Castrate	7.2±0.15	7.3±0.6**	8.13±0.41***	7.5±0.32**
RBC ($x10^3$cells/mm^3)				
Non-Castrate	9.33 ±0.176	8.9 ± 0.115	8.6 ±0.057	8.1 ± 0.066
Castrate	3.10 ± 0.078	3.5 ± 0.312**	3.6 ± 0.288***	3.4 ± 0.208****
WBC ($x10^3$cells/mm^3)				
Non-Castrate	9.14 ± 0.44	8.05 ±0.218	8.03 ± 0.034	7.90 ± 0.549
Castrate	4.6 ±0.061	5.61 ±0.337***	5.73 ± 88.19****	5.57 ± 0.463*
PLT ($x10^3$cells/mm^3)				
Non-Castrate	2.83 ±0.167	3.06 ±0.578	2.85 ±0.028	2.39 ±0.170
Castrate	1.19 ±0.056	1.28± 0.332*	1.39±0.165***	1.43 ± 0.233*
Neut (%)				
Non-Castrate	64.33±1.67	64.67±3.18	66±1.16	60.67±0.88
Castrate	63±0.58	63±2.89	64.67±2.23	72±0.58***
Lymp (%)				
Non-Castrate	33.67±1.2	34.67±3.18	31±0.58	36±2.08
Castrate	34.33±2.33	37.33±2.91	34.67±2.6	27±1.16*
Na^+ (mmol/L)				
Non-Castrate	139±1.16	133±0.58	135±1.16	134±0.58
Castrate	136±2.08	138±1.16*	134±2.08	137.67±2.03
K^+ (mmol/L)				
Non-Castrate	3.93±0.07	3.36±0.15	3.43	3.47
Castrate	3.6±0.21	3.86±0.12*	3.5	3.73
CL^- (mmol/L)				
Non-Castrate	106.67±1.67	102.33±1.45	102.33±1.45	102.67±1.45
Castrate	100±2.89	105.33±0.33	100.67±0.67	105±2.89
Urea (mg/dl)				
Non-Castrate	22±4.16	13±1.16	14.33±0.88	13.33±0.33
Castrate	26.33±4.05	29.33±0.88*	19±1.16*	27±4.04*
Creatinine				
Non-Castrate	0.6±0.05	0.4±0.05	0.53±0.03	0.36±0.03
Castrate	0.6±0.05	0.67±0.03*	0.5±0.05	0.63±0.12
Cu^{2+} (mmol/L)				
Non-Castrate	4±0.57	3±0.57	3.33±0.33	3.67±0.33
Castrate	5.27±0.09	5.2±0.21*	6.1±0.16**	5.23±0.03**
Zn^{2+} (mmol/L)				
Non-Castrate	3.33±0.88	2.67±0.33	3±0.58	3.67±0.33
Castrate	3.37±0.15	4.3±0.35*	5±0.06*	3.57±0.41

*P≤ 0.05, **P<0.01, ***P<0.001, ****P<0.0001 - significant compared with the non-castrate

DISCUSSION

The hazardous nature of general anaesthesia in ruminants makes local and regional anaesthesia more favoured in ruminants (Hall *et al*., 2001). Extradural administration of local anaesthetics and some other drugs like opioids, alpha 2 agonists, and ketamine is an established anaesthetic technique for surgeries caudal to the diaphragm (Hall *et al.,* 2001; Habibianah *et al.*, 2011). Results from this study showed that extradural lignocaine-tramadol mixture produced a longer duration of analgesia but a shorter duration of recumbency in the non-castrated compared with the castrated goats (Table 1). However, the onset of drug action was similar in both groups(Table 1). the long duration of analgesia of 83.7±20.8 min in the non-castrate goats is much longer than the established duration of about 60 minutes by plain lignocaine (Hall *et al*, 2001). This prolonged analgesia is clearly attributable to tramadol in the mixture. Furthermore, this finding is consistent with a longer duration of analgesia with tramadol-lignocaine than lignocaine alone without any side effects in previous similar studies (Natalini and Robinson, 2000; Bigham*et al.* 2010; Dehkordi*et al*, 2012; Marzok and El-Khodery, 2015). It is interesting that the duration of recumbency of the two groups of goats studied was very short (Table1). Reasons for this could be because of the absence of adrenaline which usually causes

vasoconstriction thereby prolonging the duration of action of lignocaine (Hall *et al.,* 2001).

In addition to this, about a half of the usual dosage of lignocaine was used since its use was in combination with another drug. The dosages employed in this study were as used in a previous study in goats (Dehkordi*et al.,* 2012). Furthermore, opioids are known to block only the sensory neurons and their sole use as epidural anaesthetics result in analgesia without hindlimb paralysis (Habibianah*et al,* 2011). Epidural anaesthesia may result in changes in heart rate and blood pressure as a result of sympathetic blockade (Veering and Cousins 2000). The HR, RR, and rectal temperature were not significantly different in comparison with baseline values throughout the study. This is in agreement with previous results reported following extradural tramadol in combination with lignocaine in cattle (Bigham*et al.,* 2010). Although, the castrate goats exhibited a rise and fall trend especially in the respiratory rate and rectal temperature (Fig 2, 3) which might be due to higher excitability of the castrate and the drug effects. These findings suggest that both epidural injections of tramadol and lignocaine did not produce adverse significant cardio-depressant effect in goats and further supported the safety of epidural tramadol injections as earlier reported in dogs, pigs and cattle (Alonso *et al.,* 2005; Ali *et al.,* 2010; Ajadi *et al.,* 2012). There were fluctuations in the hematological values post administration of epidural tramadol- lignocaine which might be attributed to the drug and testosterone level differences between the castrate and non-castrate. However, the reduced hematological result in the castrate has been earlier reported that reduction of normal serum testosterone levels as in castrated animals is associated with suppression of erythropoiesis (Olaifa and Akpan, 2017).

The reduction in Hb, PCV, RBC and WBC in the non-castrate could also be due to blood pooling into the reservoir like spleen as a result of the administered drugs. Copper plays an important role in body metabolism, largely because it allows many critical enzymes to function properly (Harris, 2001). Copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Cu plays a role in the production of hemoglobin, myelin, melanin and it also keeps thyroid gland functioning normally (Groff *et al.,* 1995; Harris, 2001).

Maintaining the proper dietary balance of Cu, along with other minerals such as zinc and manganese, is important (Araya *et al.,* 2006). Copper level of castrate animals was significantly higher than in intact animals at the 2nd hour post administration. This transient copper accumulation might be due to reduction in the synthesis of the copper transporter protein ceruloplasminto cause failure of excretion of copper into bile and it accumulates in body tissues and this could lead to major hepatic and neurological involvement in chronic accumulation (Bhalerao *et al.,* 2016). Serum urea level increases significantly an hour post administration in the castrate which depicts a transient suppression of urea excretion by the kidneys although there were no clinical adverse effects observed. There were fluctuations in the zinc level but not significant meaning no negative effects of the drugs on zinc metabolism.

It could be concluded from this research that epidural administration of tramadol-lignocaine combination in castrate is beneficial as the onset of action is fast and the duration of recumbency is short in both castrate and non-castrate which means quick recovery of the goats following procedures. Also, this combination is safe in castrate animals as well as in intact animals especially West African dwarf goats as there were no deleterious effects. However, further studies could combine adrenaline for prolonged recumbency when required.

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Protective Effects of Magnesium Chloride on Liver Enzymes and Biomarkers of Oxidative Stress in high fat diet fed Rats

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Summary: The excessive consumption of high cholesterol diet has been associated with an increased incidence of obesity. This is because obesity induced pathologies with high mortality, such as complications of dyslipidaemia, diabetes mellitus, arthritis, hypertension, myocardial infarction, and hepatocellular carcinoma. Although the associated, disease are enhanced by formation of oxidative stress, lipid peroxidation and hypercholesterolaemia**.** Magnesium chloride is found to be beneficial in a wide range of diseases. Magnesium is one of the most neglected mineral in human body. It is crucial for a healthy and lasting life. Magnesium is responsible for the activation of more than 300 enzymes in the body. The present study intends to determine the protective effect of magnesium chloride on liver enzyme and biomarker of oxidative stress in high fat diet fed rats. Twenty (20) adult Male Wistar rats weighing (100 – 150) grams randomly divided into three treatments and one control groups of five rats each (n = 5). Group I Normal control receive normal feed only for 6weeks, Group II received high fat diet only for 6weeks, Group III received high fat diet with 250 mg/kg for 6weeks of mgcl$_2$ and Group IV received 500 mg/kg for 6weeks of $MgCl_2$ respectively all treatments were administered via oral route, at the end of the sixth week rats were euthanized and blood samples were drawn from the heart by cardiac puncture and used to estimate oxidative stress biomarkers (Superoxide dismutase, Catalase and Glutathione peroxidase), lipid peroxidation biomarkers (Malondialdehyde) and liver enzymes. Analysis of variance and Turkey's post hoc test were used to analyze the data obtained. In relation to the liver enzyme, the showed that there was a significant ($p<0.05$) decrease in value of AST, ALT and ALP in the group co-administered with the doses of the Magnesium chloride to compared to the control. For the oxidative stress biomarkers assessed, the results showed that there was significant decrease ($P < 0.05$) in the SOD, CAT and GP_x level of the high fat diet fed groups, co-administered with 250 and 500 $MgCl_2$, when compared with the high fat diet fed group only. Also, the lipid peroxidation shows significant ($p<0.05$) decrease in the groups administered the two doses of Magnesium chloride (250 and 500 mg/kg) respectively as compared to control. In relation to the liver enzyme, the showed that there were significant ($p<0.05$) changes in value of AST, ALT and ALP in the group co-administered with the two doses of the Magnesium chloride compared to the control. The result showed that high-fat diet induces ROS, dyslipidaemia and release of biological metabolite, as evidenced by the rise in oxidative stress and activities of liver enzymes. $MgCl_2$ administration also protected the body against rise in the metabolites despite consumption of high-fat diet by the Wistar Rats.

Keywords*:* High fat diet, Liver enzymes, Oxidative stress, Magnesium chloride.

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INTRODUCTION

The first description of a 'high-fat diet' to induce obesity by a nutritional intervention was in 1959 (Masek and Fabry 1959). Subsequent studies have revealed that high-fat diets promote hyperglycemia and whole-body insulin resistance, and numerous researchers have examined their effects on muscle and liver physiology as well as insulin signal transduction. From this experience, it is generally accepted that high-fat diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised β-cell function (Oakes *et al.,* 1997; Ahren *et al.,* 1999; Lingohr *et al.,* 2002). Most studies have employed only one high-fat formula in contrast with standard chow and did not analyze the influence of the specific fat component in the model. From the sparse data comparing different high-fat diets with respect to their metabolic effects, it is generally believed that diets based on saturated fatty acids induce the typical high-fat-diet phenotype, whereas diets containing polyunsaturated ω-3 fatty acids exert beneficial effects on body composition and insulin action (Storlien *et al.,* 1991).

The excessive consumption of high cholesterol diet has been associated with an increased incidence of obesity. This is because obesity induced pathologies with high mortality, such as complications of dyslipidaemia and diabetes mellitus (Kohli *et al.,*

2010; Buettner *et al.*, 2006; Kim et al, 2011). Although the associated, disease are enhanced by formation of oxidative stress, lipid peroxidation and hypercholesterolaemia **(**Misra *et al.*, 2010) Magnesium chloride is found to be beneficial in a wide range of diseases but one of the most neglected mineral in human body. It is crucial for a healthy and lasting life and is responsible for the activation of more than 300 enzymes in the body. Excessive accumulation of body fat is one of the leading causes of death worldwide. Paststudieshave shown that dietary modifications such as low fat diets, high-fiber diets, diets rich in flavonoids and phenolic acids can reduce metabolic syndrome risk factors (Minich and Bland, 2008; Lyer *et al.*, 2009).

Cholesterol is a soft waxy substance found in animal cell membranes used for the synthesis of digestive bile acids, vitamin D and certain steroid hormones and in plant it is known as phytosterols which are believed to compete with cholesterol for absorption in the intestines (Ostlund et al.,2003; Weingartner et al.,2008). High density lipoprotein cholesterol (HDL) also known as good cholesterol which picks up excess cholesterol dropped off by low density lipoproteins, and transports it to the liver for excretion. High amount of HDL is usually of more health significance than the LDL cholesterol (Lewis and Rader2005). Since HDL helps remove cholesterol from the blood, it thus, keeps cholesterol from building up in the arterial walls, Low density lipoprotein cholesterol (LDL) also known as bad cholesterol is the major blood cholesterol carrier from the liver to the tissues. It transports lipids such as phospholipid and triglyceride within the extracellular fluids. Too much of LDL cholesterol in the body can lead to the build-up of plaque on the arterial walls (Tymoczko et al., 2002).

Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation (Rude 2012). It was reported that Mg supplementation has beneficial effects on blood levels of HDL-C (Djurhuus et al.,1999), cholesterol and/ or triglycerides (Guerrero-Romero et al., 2000). Oral intake of magnesium also has beneficial effects on lipid metabolism and efficiency of insulin in maintaining glucose homeostasis in human subjects (Saris et al., 2000; Barbagallo 2003). Mg deficiency is known to decrease the level of GSH in erythrocytes (Hsu et al., 1982; Weglicki et al., 1996) and even inhibit its biosynthesis (Mills et al., 1984), and in agreement with these findings, magnesium supplementation was shown to induce a significant increase in GSH in kidney of mice treated with cadmium (Djukić-Ćosić et al., 2007). Magnesium intake is capable of decreasing the blood concentration of vanadate in rats (Ścibior et al., 2012) and the cadmium level in blood, kidney, spleen, and bone marrow in rabbits (Bulat et al., 2008). In addition, both oral and intraperitoneal supplementation of magnesium acetate were effective against cadmium toxicity (Matović et al., 2012). Other findings suggest that magnesium chenodeoxycholic acid (Mg-CUD) may prevent liver fibrosis induced by CCl_4 (Kang et al., 2012). Moreover, magnesium has been shown to have protective effects against oxidative stress observed in experimental animals with different pathologies (Hans et al., 200; Zhang et al., 2003). Magnesium supplementation appears to attenuate the hepatotoxicity of CCl_4 as reported by Eidi et al. (2014) and prevent liver fibrosis ((Kang et al., 2012). The nephroprotective effect of Magnesium has also been well documented (Bulat et al., 2008) and seems to be related to its property of scavenging free radicals before the occurrence of damage to cellular macromolecules.

The aim of this research is to investigate the protective effects of Magnesium chloride on liver enzymes and biomarkers of oxidative stress in rats fed on high fat diet.

MATERIALS AND METHODS

Chemical used

All chemicals were obtained commercially and were of analytical grade: (Cholesterol: Sigma chemical Company St. Louis USA) and Magnesium Chloride (Sigma Aldrich).

Animals and induction of Diabetes

Wistar rats, weighing between 100 – 150g, were used for the study. They were bred and purchased in the animal house of the Department of Human Physiology, Ahmadu Bello University (ABU), Zaria and according to the Principle of Laboratory Animal Care, ABU, Zaria, Nigeria. The animals were kept in well-aerated laboratory cages at room temperature (25-26°C) in the animal house. They were fed with growers' and starters' mash (Vital Feeds Company, Kaduna, Nigeria), and given access to drinking water during the stabilizing period. High fat diet diet was induced by feeding the rats with standard animal feed + high- fat diet (10% groundnut oil, 20% groundnut meal and 2% cholesterol) according to Kolawole et al, (2012) with slight modification. The animals were fed with the high fat diet for a period of eight (8) weeks.

Experimental design

Twenty (20) Wistar rats weighing between 100g-150g were used for the study. The rats were randomly divided into 4 groups of five (n = 5) animals in each

Group 1: Normal control received normal feed only

Group 2: High fat diet control untreated received high fat diet only, for a period of six weeks

Group 3: High fat diet + 250mg/kg $MgCl_2$ orally for a period of six weeks (Ige *et al.*, 2016)

Group 4: High fat diet + 500mg/kg $MgCl_2$ orally for a period of six weeks

Blood Sample Collection and Serum Preparation
At the end of the six weeks of administration period, the rats were euthanized by cervical dislocation and blood samples were collected from the animals through cardiac puncture. About 5 mL of blood were collected into specimen bottles and allowed to clot and separated by centrifugation at 3,000g for 10 minutes using Centrifuge Hitachi (Universal 32). The supernatant obtained were used for the determinations of oxidative stress biomarkers and liver enzyme.

Determination of Liver Enzyme Activity
Activities of serum alanine amino transaminase (ALT) was estimated by method adopted by Tietz (I995), aspartate amino transaminase (AST) was determined by method of Henderson and Moss (2001), while alkaline phosphatase (ALP) was determined according to the method of Scherwin (2003). All tests were carried out using ELITECH clinical system kits.

Determination of Biomarkers of Oxidative Stress assay:

Superoxide Dismutase Activity
Activity of SOD in the rat serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The assay kit is based on the principle of superoxide inhibition of auto oxidant of hematoxylin as described by Martin et al. (1987).

Catalase Activity
Catalase (CAT) activities were assayed by the method of Sinha (1972). 0.1 mL of Plasma and 1.5 mL of phosphate buffer were added. To this, 0.4 mL of hydrogen peroxide was added and the reactions were arrested after 30 and 60 second by the addition of 2.0 mL dichromate acetic acid reagent. A control was also carried out simultaneously. All the tubes were heated in a boiling water bath for exactly 10 min, cooled and absorbance read at 620 nm. Standards in the range of 2-10 mmoles were taken and processed as the test. The activities of catalase were expressed as μmoles of hydrogen peroxide consumed/min/mg of protein (unit per milligram of protein).

Glutathione Peroxidase
The NWLSSTM Glutathione Peroxidase Assay kit was used which is an adaptation of the method of Paglia and Valentine (1967). Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG was then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ (resulting in decrease absorbance at 340nm) and recycling the GSH. Since GPx is limiting, the decrease in absorbance at 340nm is directly proportional to the GPx concentration. The absorbance was read at 1,2 and 3 minutes against reagent blank. The absorbance for blank was subtracted from the sample reading to give the corrected value. Thus, GPx activity was calculated using 8.412 as the extinction coefficient:

$GPx(U/L) = 8.412 \times \Delta A\ 340/min$

U/L = unit activity per liter

Δ A 340/min = change in absorbance at 340 per minute.

Lipid peroxidation biomarker (MDA)
Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substances method (Gallou *et al*., 1993). Plasma malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hadley (1990) using Malondialdehyde Assay kits from Northwest Life Sciences Specialities (NWLSSTM, product NWK-MDA01). Butylated hydroxytoluene (BHT) in methanol reagent was used as the control. The method is based on the spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA at 532nm. The MDA formed will therefore be quantified using an extinction coefficient of 1.56 x 10_5/mole/cm (Yagi, 1987). The amount of MDA formed in the control samples is subtracted from the amount in the experimental samples to obtain the amount of MDA in each sample. Since absorbance is directly proportional to the concentration, thus; concentration of MDA in each sample = Absorbance in sample – Absorbance in control x 10_5 nmol/ml ÷ 1.56 x 10_5 $M^{-1}CM_1$

Statistical Analysis
The data obtained were expressed as mean ± standard error of mean (SEM) and data were statistically analyzed using analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The values of $p \leq 0.05$ were considered as significant.

RESULTS

Liver enzyme assay

Aspartate aminotransferase (AST): Figure 1 shows a significant($p<0.05$) increase in the group that were fed on high fat diet alone, as compared with the group that was fed on normal feed. However, administration of 250 mg/kg mGcL2 significantly decrease ($p<0.05$) the AST level as compared to the high fat diet control group. Consequently, 500 mg/kg $MgCl_2$ significantly increase the activity of the enzymes as compared to the high fat diet control group.

Alanine Amino Transferase (ALT) and Alkaline phosphatase: Figure 2 shows a significant decrease in the group that were fed on high fat diet as compared with the control with the normal control group. With regards to the 250 mg/kg $MgCl_2$ significantly increase($p<0.05$) the levels of the ALT. In relation to the 500 mg/kg $MgCl_2$ there was a significant increase as compared to the high fat diet control group.

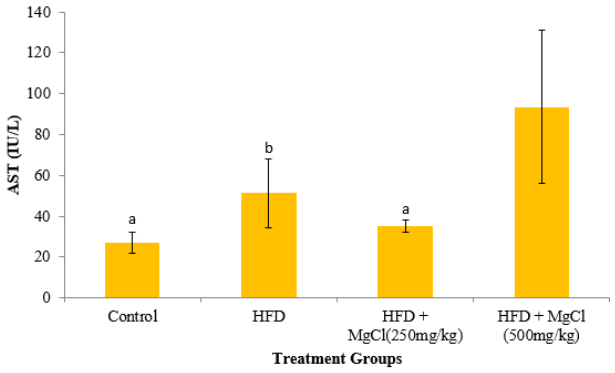

Figure 1:
Effect of co-administration of magnesium chloride and high-fat diet on serum AST level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a, b) differ significantly (P < 0.05).

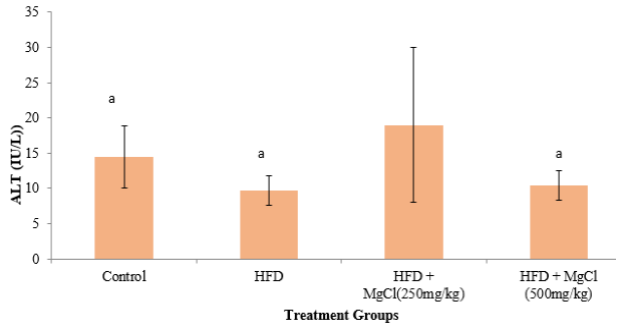

Figure 2:
Effect of co-administration of magnesium chloride and high-fat diet on serum ALT level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a, b) differ significantly (P < 0.05).

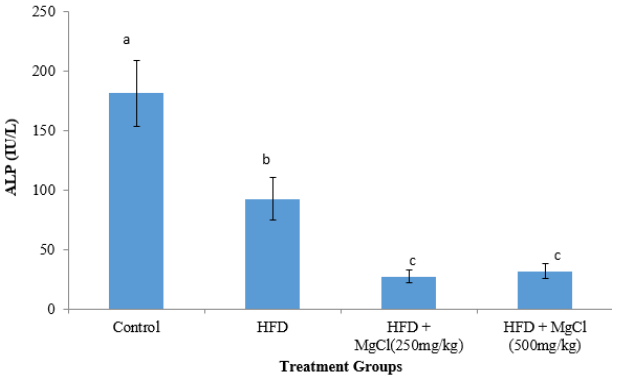

Figure 3:
Effect of co-administration of magnesium chloride and high-fat diet on serum ALP level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly (P < 0.05).

Alkaline Phosphatase (ALP)
In figure 3, a significant ($p \leq 0.05$) increase in values of alkaline phosphatase (ALP) obtained in the group that was fed on normal feed when compared to high fat diet control. Administration of 250 and 500 mg/kg $MgCl_2$ significantly ($p<0.05$) decreases the ALP level as compared to the high fat diet control group.

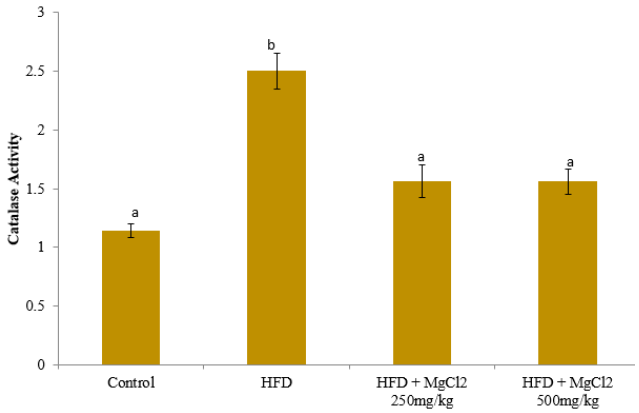

Figure 4:
Effect of co-administration of Magnesium chloride and high-fat diet on serum Catalase level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly (P < 0.05).

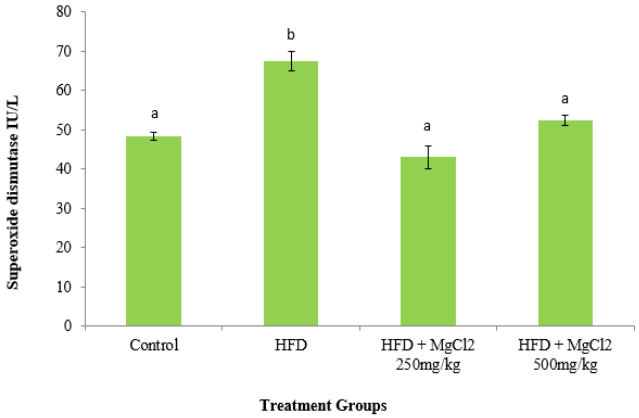

Figure 5:
Effect of co-administration of magnesium chloride and high-fat diet on serum Superoxide dismutase level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly (P < 0.05).

Antioxidant enzyme assay:

Catalase activity (CAT): CAT activities in the magnesium chloride co-administered with high fat diet, control group alone and HFD group only are shown in figure 4. 250 mg/kg and 500 mg/kg magnesium chloride co-administered with high fat diet showed significant ($p < 0.05$) decrease in CAT activity with values of 1.56 ± 0.14 IU/L and 1.56 ± 0.11 IU/L when compared to the high fat diet group only with a value of 2.50 ± 0.15 respectively.

Superoxide dismutase (SOD): Figure 5 shows the activity of SOD in the magnesium chloride administered with HFD, control group alone and high fat diet group only. Magnesium chloride co-administered with cholesterol diet showed significant

($p < 0.05$) decrease in SOD activity of 250 mg/kg and 500 mg/kg magnesium chloride with values of 43.00 ± 2.81 IU/L and 52.40 ± 1.36 IU/L when compared to the high fat diet group only with a value of 67.40 ± 2.48 IU/L respectively. The result shows that the activity of SOD was decreased despite consumption of high fat diet in groups treated with magnesium chloride.

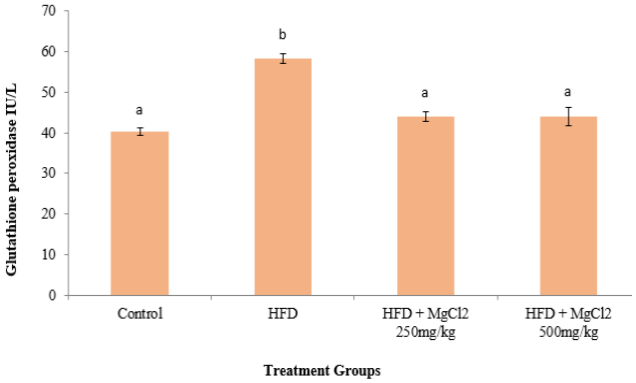

Figure 6:
Effect of co-administration of Magnesium chloride and high-fat diet (HFD) on serum Glutathione peroxidase level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differs significantly ($P < 0.05$).

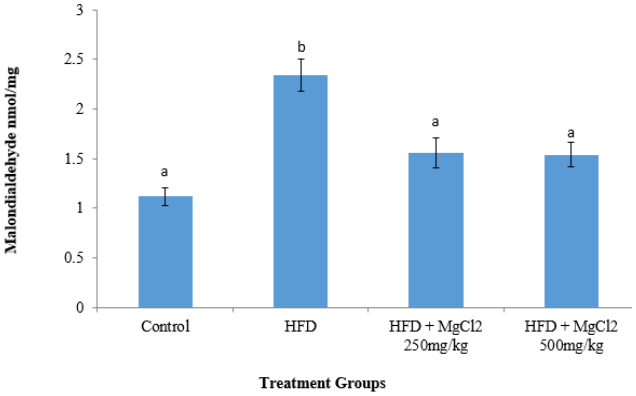

Figure 7:
Effect of co-administration of Magnesium chloride and high-fat diet on serum Malondialdehyde concentration level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly ($P < 0.05$).

Glutathione peroxidase activity (GPx): Figure 6 shows the activity of GPx in the magnesium chloride administered with high fat diet and high fat diet group only. Magnesium chloride co-administered with high fat diet revealed a significant ($p < 0.05$) decrease in GPx activity with a value of 44.00 ± 1.30 IU/L for 250 mg/kg magnesium chloride and 44.00 ± 2.17IU/L for 500 mg/kg magnesium chloride when compared to the high fat diet group only with a value of 58.40 ± 1.21 IU/L respectively. Magnesium chloride administration ameliorates GPx activity despite consumption of high fat diet.

Malondialdehyde (MDA) Concentration: Figure 7 shows the activity of MDA in the magnesium chloride administered with high fat diet, control group alone and high fat diet group only. high fat diet significantly ($P< 0.05$) elevated the serum MDA level when compared with the normal control animals. Magnesium chloride at doses of 250 mg/kg and 500 mg/kg significantly ($P < 0.05$) reduced the serum MDA levels with values of 1.56 ± 0.15IU/L and 1.54 ± 0.12 when compared with the high fat diet group only with a value of 2.34 ± 0.16IU/L respectively, despite the consumption of high fat diet.

DISCUSSION

Metabolic disturbances both in obese and experimental animals, obesity has been shown to be accompanied by an increase in oxidative stress markers (Diniz *et al.*, 2006). An augmented oxidative stress, if coupled with an attenuated antioxidant capacity tends to disrupt the normal redox homeostasis leading to irreversible damage to membranes and other macromolecules (Kamata and Hirata, 1999; Levine and Stadtman, 2001). High fat diet administration is routinely employed in experimental models of obesity and metabolic syndrome. It has been argued that excessive accumulation of fat leads to enhanced production of Reactive oxygen species in adipocytes and systemic tissues (Furukawa *et al.*, 2004). Obesity, insulin resistance and hyperglycemia, develop over a period of several weeks of Hhigh fat diet administration and it has been demonstrated that increased Oxidative stress precedes these changes (Matsuzawa *et al.*, 2008). Furthermore, an increase in liver biomarkers such as AST, ALT, and ALP in the plasma of rats fed with high cholesterol dietcould be an indication of liver damage resulting in the injury of hepatocytes which may have caused a leakage of cytosolic enzymes (AST, ALT, and ALP) from the cell into circulation, thus, leading to an increase in the levels of these enzymes in the plasma (Pratt and Kaplan, 2000). The result shows a reduction in the function of liver biomarker enzymes due to the increase in AST, ALT, and ALP levels as compared to the HFD control. Supplementing with magnesium chloride caused a significant decrease in plasma AST, ALT and ALP levels when compared with the control ($P<0.05$). Generally, hypercholesterolemia is considered to be an increase in both the abnormal hepatic and serum cholesterol and triglyceride levels (Wang et al., 2010). The administration of dietary cholesterol has been shown to influence hepatic lipid metabolism in rats (Wang et al., 2010). Also, an increase in serum total cholesterol may result in impairment of triglyceride metabolism which causes deposition or accumulation of free fatty acids in the liver, thereby leading to a condition otherwise known as fatty liver (Wang et al., 2010). This expanded liver

fatty acid pool results in an increase in peroxisomal and mitochondrial β-oxidation which leads to the formation of reactive oxygen species. This may, in turn, result in the progression of liver injury via the process of a local proinflammatory state (Schwimmer et al., 2008). Hence, our result showed that magnesium chloride is able to protect the liver from oxidative damage due to its phenolic contents. This work agrees with the findings of Adekiya *et al.* (2018).

The oxidative stress biomarkers, superoxide dismutase, glutathione peroxidase, and catalase activity groups fed with high fat diet expressed the highest level of activity as compared with the control groups. There was a statistical significant ($p<0.05$) decrease in superoxide dismutase activity as compared to control. Similar observations with a corresponding decrease in enzymatic antioxidants (SOD and GPx) have been reported in number of studies on an HFD (Noeman et al., 2011; Rahman et al., 2017). The groups administered with 250mg/kg and 500mg/magnesium chloride. Had an equal increase in activity, this shows that the activity of oxidative biomarkers is not dose dependent. This result agrees with the finding of Halliwell, (2007). Who demonstrated an increase in biomarkers of oxidative stress with administration of substance that cause release of free radicals in the body.It has been reported that hypercholesterolemia enhanced the production of oxidative stress and increased Lipid peroxidation (LPO) (Cox and Cohen, 1996). Studies have shown that a diet rich in high cholesterol concentration results in an increase in the levels of LPO by free radicals and aggravates hypercholesterolemia (Lee et al., 2006). The increase in cholesterol diet also caused a marked elevation in the levels of plasma MDA; an initial outcome of LPO. However, an observable decrease in the levels of plasma MDA of hypercholesterolemic rats treated with magnesium chloride clearly indicates a great significant regulation of cholesterol metabolism by lowering the MDA level. Therefore, magnesium chloride supplementation can be considered as important antioxidant therapeutic diet in hypercholesterolemic state; due to their great significant regulatory effect in the plasma cholesterol concentration by lowering the plasma MDA which in turn results in the inhibition of oxidative stress. Superoxide (SOD) is an antioxidant enzyme that catalyzes the conversion of two superoxides into H_2O_2 and oxygen. It acts as a major defense system against the cytotoxic effects of superoxide radicals (Caldwell *et al.,* 2008). SOD is metal-containing enzyme that depends on bound trace metals for antioxidant activity. They are of two types: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD and each type of SOD plays a different role in keeping cells healthy. Different isoforms of SOD are located at different sites within the cells (Caldwell *et al.,* 2008). Bohr *et al.* (2004) showed a significant increase in small intestine SOD activity of diabetic rats. Increased activity of SOD in the brain of diabetic rats has also been reported (Genet *et al.,* 2002). Decrease in the activity of SOD in diabetes could possibly be a response to increased generation of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation (Pari and Latha, 2004). Kumawat *et al.* (2005) has also reported that the reduced activity of SOD in the erythrocytes of diabetic rats could be due to ageing or an increase in the glycation of SOD.

With regards to the catalase there was a significant increase in the group that was fed on high fat diet. However, administration of Magnesium chloride at the doses tested (250 and 500 mg/kg significantly decrease the levels. Catalase (CAT) is an antioxidant enzyme that is produced naturally in the body and found in peroxisomes in eukaryotic cells. It is particularly important in conditions where glutathione (GSH) is limited or the activity of GPx is diminished (Caldwell *et al.,* 2008). CAT allows for important cellular processes which produce H_2O_2 as a by-product to occur by preventing excessive buildup of hydrogen peroxide and also protect against hydrogen peroxide mediated oxidative damage. In the small intestine, CAT activity was significantly increased in the diabetic rats (Bohr *et al.,* 2004). CAT activity has been shown to be significantly high in diabetic patients (Kumawat *et al.,* 2005) The uncontrolled generation of H_2O_2 as a result of the auto-oxidation of glucose, protein glycation and lipid oxidation in diabetes is markedly responsible for the decline in catalase activity (Saravanan and Ponmurugan, 2012).

In relation to the glutathione peroxidase, administration of 250 and 500mg/kg magnesium chloride significantly decrease in the level as compared with the control. Glutathione peroxidase (GPx) is a group of enzymes of which most contain selenium. It helps to protect the cell from damage due to free radicals like hydrogen and lipid peroxides and its actions take place in the presence of glutathione, the master antioxidant. They act like catalase by degrading hydrogen peroxide. GPx metabolizes hydrogen peroxide to water with the usage of reduced glutathione as a hydrogen donor (Caldwell *et al.,* 2008). They also reduce organic peroxides to alcohols, providing another way for the removal of toxic oxidants. A decrease in the activity of GPx in the pancreas of diabetic rats has also been reported (Babujanarthanam *et al.,* 2011). Reduced activity of GPx could be due to low content of glutathione in diabetic state, since glutathione serves as a substrate and cofactor of GPx (Saravanan and Ponmurugan, 2012). Decrease in GPx activity could be a result of a number of deleterious effects due to the accumulation of toxic products (Saravanan and Ponmurugan 2012).

The outcome of this present study suggests that magnesium chloride is able to protect the liver from oxidative damage. It also revealed that the treatment of

hypercholesterolemic rats with magnesium chloride inhibited the generation of MDA in the plasma, which in turn resulted in the formation of lipid peroxidation. Additionally, the scavenging activities and the hypocholesterolemic effects of magnesium chloride after the administration of high cholesterol diet were also established by the study.

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Sexual Dimorphism in Osteometric Indices of Kuri Cattle Skulls

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Summary: This work investigated 30 skulls of the Kuri cattle comprising 15 males and 15 females, of three age groups, 10 young (9 months to less than 3 years), 10 middle aged (3 to 5 years) and 10 aged (greater than 5 years). The skulls were prepared using hot water maceration technique. Using Ruler, thread and divider; 59 Osteometric parameters were taken to determine sexual dimorphism, only 6 indices showed statistically significant differences between the sexes. These indices were maximum intercondylar width (MICW), right supraorbital foramen to interfrontal suture (ISRSOF), left supraorbital foramen to interfrontal suture (ISLSOF), lateral intercornual length (LICL), intertemporal line width (ITLW) and horn base circumference (HBC). The female had longer viscerocranial length (VCrL) both on the nasal and palatal aspects, but were wider in the male. The male had longer and wider neurocranium. The paracondylar process length (PCPL) was longer in the female, but the male had wider interparacondylar width (IPCW) and maximum intercondylar width (MICW) While the ISRSOF significant difference appeared only at the middle-age group at $p<0.05$, the ISLSOF did not differ significantly at any particular age group but only overall mean of all the three age groups (n=15) presented the difference significantly. The MICW showed significant difference ($p<0.05$) at two age groups; young and the middle-age. The LICL and ITLW values are highly significant ($p<0.01$) and the HBC value was also significant ($p<0.05$) all at the middle age. No significant difference was recorded between the two sexes in the aged Kuri cattle; indicating that the female tends to progressively increase in size beyond 5 years old thereby making these values insignificant from the male counterpart at old age. In conclusion, the middle-age Kuri cattle have the most sexually dimorphic osteometric landmarks in the skull with the male having higher values than the female. This data will be useful for anatomical, developmental, anthropological forensic and clinical studies, and form basis for comparison with other breeds of cattle.

Keywords*:* Osteometry, Kuri cattle, Skull, Age, Sex dimorphism

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INTRODUCTION

The Kuri (White lake cattle or Buduma) is a breed of cattle found mainly on the Islands and along the shore of the Lake Chad, formed by the four neighbouring countries of Nigeria, Niger, Cameroon and Chad considered to be the area of origin of the breed (Malbrant *et al.* 1947a and Malbrant *et al.* 1947b). The Kuri breed is of the Hamitic long horn (*Bos taurus longifrons*). The actual population of Kuri cattle has never been precisely known, however, several authors put the worldwide estimate within the range of one hundred to four hundred thousand (Tawah *et al.* 1997). The Kuri breed is an imposing breed, whose size is rarely seen in African cattle with well-developed bones (Epstein, 1971). The typical characteristic of the Kuri is the disproportionate large horns that draw immediate attention, coupled with the lack of a hump adds to the compact appearance of the Kuri cattle. Its height and size contribute to the average weight of 550kg, but under feedlot, can reach up to 600-700kg. (Epstein, 1971; Queval *et al.* 1971).

Sexual dimorphism in skulls of different animal species is of importance in anatomic, archaeological and forensic studies (Bornholdt *et al.* 2008). Even though classical differences can be observed phenotypically in the skull of some species between sexes (Yahaya *et al.* 2012a), this can be marginal in some. This requires precise morphometric analyses to differentiate them (Mazak, 2004).

Studies have been carried out on the Kuri cattle in the area of production and distribution (Epstein, 1971; Malbrant *et al.* 1947a and b; Tawah *et al.* 1997), dental abnormalities (Gambo *et al.*, 2015a), mandibular and maxillofacial regions (Gambo *et al.* 2015b; Gambo *et al.* 2016). In our continuous effort to document the anatomy of the skull of Kuri cattle, in this work we studied the different osteometric indices in the skull of Kuri cattle and report herein the skull osteometric indices of the male and female Kuri cattle.

MATERIALS AND METHODS

A total of thirty (30) Kuri cattle consisting of 15 males and females each were used for this study. The breed was identified by their typical characteristics such as the disproportionate large horns, coupled with the lack of a hump and color. This included 10 young Kuri cattle between the ages of 9 months to less than 3 years, 10 middle-aged between the ages of 3 to 5 years old, and 10 Aged Kuri cattle greater than 5 years all consisting of 5 male and female in each group. The animals were physically examined for their state of health particularly for absence of musculoskeletal deformities, and age was estimated based on dental eruption technique as described by Pasquini (1982). Sex of each animal was determined by physical observation of the perineum and inguinal areas. The heads were obtained immediately after slaughter from Shomolu Bariga, Bodija, Ijebu-Igbo and Maiduguri Main abattoirs of Lagos, Oyo, Ogun and Borno states of Nigeria respectively. The skulls were prepared according to the hot water maceration technique described by Olopade and Onwuka (2005), Yahaya *et al.* (2012b) and Gambo *et al.* (2015b). A digital camera with 16 megapixels lens was used in the production of figures in this study.

A total of 59 osteometric parameters were obtained on each skull with the aid of metric rules, vernier calipers, threads and a pair of dividers and compasses and depicted in figures 1-5. Morphometric parameters were adopted from the works of Olopade and Onwuka (2005) and Yahaya *et al.* (2012b). Vital landmarks were produced to fit this species/breed.

Osteometric measurements and indices

1. Incisive – Nuchal length (**INL**): Maximum dimensions of the skull when laid on an even surface from the rostral tip of the incisive bone to the caudal level of the nuchal protuberance.
2. Supraorbital width (**SOBW**): Maximum distance between the dorsomedial edges of the orbit at the frontal bone.
3. Intermedial canthi length (**IMCL**): Distance between the two medial canthi.
4. Interlateral canthi length (**ILCL**): Distance between the two lateral canthi.
5. Interfrontal suture length (**IFSL**): Maximum length of the interfrontal suture.
6. Interfrontal suture to right supraorbital foramen (**ISRSOF**): Distance between right supraorbital foramen to interfrontal suture directed medial.
7. Interfrontal suture to left supraorbital foramen (**ISLSOF**): Distance between left supraorbital foramen to interfrontal suture directed medial.
8. Intercornual protuberance to line of intersection of Right (**ICPLIR**): Distance between intercornual protuberance to line of intersection.
9. Intercornual protuberance to line of intersection of left (**ICPLIL**): Distance between intercornual protuberance to line of intersection of 5 above.
10. Nasal length at midline (**NLM**): Maximum length of nasal bone along the midline.
11. Nasal length at lateral prongs (**NLLP**): Maximum length of nasal bone at the level of the lateral nasal prongs.
12. Nasal width (**NW**): Maximum width of the two nasal bones.
13. Viscerocranial length (**VCrL**): Maximum distance from nasofrontal suture to the rostral end of the interincisive suture along the median plane.
14. Interpalatine suture length 1 (**IPSL1**): Maximum length of the horizontal parts of palatine at the suture.
15. Interpalatine suture length 2 (**IPSL2**): Maximum length of the horizontal parts of palatine process of the maxilla at the suture.
16. Interpalatine suture length (**IPSLM**): Maximum length of the horizontal parts of palatine and palatine process of maxilla at the suture.
17. Interincisive suture length (**IISL**): Maximum distance from mid-rostral tip of the incisive bone to the incisive-maxillary junction at the palatal surface.
18. Left Incisive fissure length (**LIFL**): Maximum rostro-caudal distance of the incisive fissure.
19. Right Incisive fissure length (**RIFL**): Maximum rostro-caudal distance of the incisive fissure.
20. Left Incisive fissure width (**LIFW**): Maximum width of the incisive fissure.
21. Right Incisive fissure width (**RIFW**): Maximum width of the incisive fissure.
22. Hard palate length (**HPL**): Maximum distance at the midline of the caudal end of hard palate to the most rostral margin of the incisive bone.
23. Palatine width at premolar 1 (**PWP1**): width between the median aspects of alveoli at premolar one.
24. Palatine width at molar 1 (**PWM1**): width between the median aspects of alveoli at molar one.
25. Palatine width at molar 3 (**PWM3**): width between the median aspects of alveoli at molar three.
26. Choana length (**ChL**): Distance between the basisphenoid to the most caudal margin of the hard palate.
27. Choana width 1 (**ChW1**): Distance between the medial margins of the two pterygoid crests.
28. Choana width 2 (**ChW2**): Distance between the medial margins of the pterygoid hamulus.
29. Maximum zygomatic width 1 (**MZW1**): Maximum distance between the zygomatic arches at the level of the orbit.
30. Maximum zygomatic width 2 (**MZW2**): Maximum distance between the zygomatic

arches at junction of zygomatic process of temporal bone and temporal process of zygomatic bone.

31. Incisivo-occipital length (**IOL**): Maximum distance between the most rostral end of the incisive and caudal limits of the external occipital protuberance.
32. Condylobasal length (**CBL**): Length of the skull measured from the caudal surface of the occipital condyles to the rostral tip of the incisive bones.
33. Intercornual Protuberance to Foramen magnum (**IPFM**): Distance between intercornual protuberance to dorsal brim of foramen magnum at the midline.
34. Foramen magnum height (**FMH**): Mid-vertical height of the foramen magnum.
35. Foramen magnum width (**FMW**): Width of the foramen magnum at the median protuberance of the two condyles in to the foramen magnum.
36. Foramen magnum circumference (**FMC**): length of the foramen magnum circumference.
37. Foramen magnum Index (**FMI**): $\frac{\text{FMH X 100}}{\text{FMW}}$
38. Maximum intercondylar width (**MICW**): Maximum distance between the two occipital condyles at the caudal surface.
39. Interjugular process width (**IJPW**): Maximum distance between the tips of the two jugular processes.
40. Paracondylar process length (**PCPL**): Distance between tips of the paracondylar (jugular) process directly to its junction with squamous occipital bone.
41. Interparacondylar width (**IPCW**): The widest breath between the most lateral ends of the paracondylar process.
42. Whole skull height 1 (**WSH1**): Distance between the most ventral part of the skull (without mandible) and the highest point of the skull when laid on an even surface.
43. Whole skull height 2 (**WSH2**): Distance between the most ventral part of the skull (without mandible) to the intercornual protuberance.
44. Skull height (**SH**): Distance between the highest point of the skull (intercornual protuberance) and the base of the mandible.
45. Intercornual protuberance to external occipital protuberance (**IPEOP**): Contour length of the distance between the Intercornual protuberance to external occipital protuberance.
46. Neurocranial volume (**NCV**): The volume of the neurocranium in milliliters. Measured by blocking all the foramina (with fresh plasticine) leading to the neurocranium except the foramen magnum. The neurocranium was then filled with fine quality millet grains, which was then emptied into a measuring cylinder to determine the volume.
47. Medial intercornual length (**MICL**): Distance between the two cornual processes at the fronto-cornual junction.
48. Lateral intercornual length (**LICL**): Distance between the two lateral edges of the fronto-cornual junction.
49. Left horn curvature length rostral (**LHCLR**): maximum curvature length of the left horn at the rostral surface.
50. Right horn curvature length rostral (**RHCLR**): maximum curvature length of the right horn at the rostral surface.
51. Left horn curvature length caudal (**LHCLC**): maximum curvature length of the left horn at the caudal surface.
52. Right horn curvature length caudal (**RHCLC**): maximum curvature length of the right horn at the caudal surface.
53. Left horn curvature length medial (**LHCLM**): maximum curvature length of the left horn at the medial surface.
54. Right horn curvature length medial (**RHCLM**): maximum curvature length of the right horn at the medial surface.
55. Left horn curvature length lateral (**LHCLL**): maximum curvature length of the left horn at the lateral surface.
56. Right horn curvature length lateral (**RHCLL**): maximum curvature length of the right horn at the lateral surface.
57. Intercornual tip width (**ICTW**): Distance between the tips of the two horns.
58. Intertemporal line width (**ITLW**): Distance between the two temporal lines at the frontal bone.
59. Horn base circumference (**HBC**): Length of circumference of the base of the horn.

Statistical Analysis

Mean values and standard deviation were obtained for each parameter; independent student *t* test was used to analyze gender difference between groups using SPSS software package.

RESULTS

The osteometric data obtained for males and females Kuri cattle are shown in Tables 1-4. In this study, only 6 out of the 59 skull parameters taken showed statistical significance. ISRSOF, HBC, MICW were significant ($p<0.05$) between sexes, while LICL and ITLW were highly significant ($p<0.01$) all at the middle age, but MICW is also significant at young age $p<0.05$ (Table 5). The overall mean (n=15 for each sex) of ISLSOF was significant ($p<0.05$), but did not show any significance at particular age group. The values of the nasal length (NLM and NLLP) and viscerocranium are higher in the female, but the nasal width (NW) is higher in the male. The neurocranial

Table 1- Skull measures and indices of the of Kuri cattle (appreciable from a Dorsal view)

Measurements (cm)	Male	Female
INL	50.38 ± 6.75	49.87 ± 4.75
SOBW	15.96 ± 1.88	14.71 ± 1.50
IMCL	14.97 ± 2.34	14.87 ± 1.74
ILCL	22.10 ± 2.52	20.53 ± 2.04
IFSL	23.63 ± 3.10	22.53 ± 2.17
ISRSOF	6.81 ± 9.12	5.83 ± 0.68*
ISLSOF	6.86 ± 0.05	5.94 ± 0.73*
ICPLIR	12.40 ± 1.98	12.88 ± 1.80
ICPLIL	12.47 ± 1.99	13.03 ± 1.61
NLM	18.59 ± 2.31	18.85 ± 2.45
NLLP	18.29 ± 2.46	18.70 ± 2.04
NW	6.29 ± 0.78	5.88 ± 0.77
VCrL	25.75 ± 2.92	26.01 ± 2.57

*Mean difference is significant at p<0.05

Table 2- Skull measures and indices of the of Kuri cattle (appreciable from a ventral view)

Measurements (cm)	Male	Female
IPSL1	6.95 ± 1.14	6.64 ± 0.91
IPSL2	9.63 ± 1.27	10.11 ± 1.21
IPSLM	16.58 ± 2.05	16.75 ± 1.81
IISL	10.66 ± 1.24	10.75 ± 1.14
LIFL	6.70 ± 0.70	6.37 ± 0.58
RIFL	6.71 ± 0.71	6.36 ± 0.56
LIFW	1.63 ± 0.20	1.57 ± 0.50
RIFW	1.65 ± 0.25	1.54 ± 0.12
HPL	27.24 ± 2.83	27.50 ± 2.43
PWP1	6.77 ± 0.99	6.95 ± 0.84
PWM1	7.94 ± 0.98	7.89 ± 0.86
PWM3	7.67 ± 0.93	7.59 ± 0.61
ChL	10.52 ± 1.42	10.10 ± 0.94
ChW1	3.30 ± 0.44	3.51 ± 0.43
ChW2	3.08 ± 0.46	3.00 ± 0.53
MZW1	19.95 ± 2.19	18.78 ± 1.46
MZW2	19.13 ± 1.74	18.37 ± 1.15
IOL	47.87 ± 4.61	46.83 ± 3.23
CBL	47.07 ± 4.16	46.62 ± 3.26

Table 3- Skull measures and indices of the of Kuri cattle (appreciable from caudal and occipital view)

Measurements (cm)	Male	Female
IPFM	10.43 ± 1.23	10.13 ± 0.67
FMH	3.95 ± 0.32	3.97 ± 0.32
FMW	3.63 ± 0.34	3.85 ± 0.38
FMC	13.75 ± 1.16	13.55 ± 0.74
FMI ^{oo}	109.23 ± 10.88	103.70 ± 11.31
MICW	10.87 ± 0.85	9.99 ± 0.84*
IJPW	8.26 ± 1.47	8.17 ± 0.88
PCPL	6.01 ± 0.72	6.17 ± 0.54
IPCW	15.35 ± 1.76	14.88 ± 1.34
WSH1	18.38 ± 2.17	17.51 ± 1.51
WSH2	16.07 ± 1.61	15.43 ± 1.25
SH	27.05 ± 3.05	26.69 ± 2.24
IPEOP	9.21 ± 1.13	8.42 ± 1.00
NCV $_{ml}$	496.47 ± 68.48	459.60 ± 29.94

*Mean difference is significant at p<0.05, ml= millilitres, ^{oo} = Parameter without unit

Table 4: Skull measures and indices of the of Kuri cattle (appreciable from frontal and caudal skull view)

Parameters (cm)	Male	Female
MICL	11.54 ± 1.60	12.45 ± 1.87
LICL	25.99 ± 3.86	23.39 ± 2.53*
LHCLR	55.50 ± 22.77	48.85 ± 21.79
RHCLR	55.55 ± 22.74	49.25 ± 21.55
LHCLC	62.21 ± 24.33	54.83 ± 24.53
RHCLC	62.26 ± 24.39	55.09 ± 24.17
LHCLM	55.69 ± 21.61	49.57 ± 21.10
RHCLM	55.53 ± 21.28	49.83 ± 20.82
LHCLL	60.53 ± 23.97	53.17 ± 23.86
RHCLL	60.48 ± 23.51	49.46 ± 24.28
ICTW	65.55 ± 20.78	65.07 ± 21.89
ITLW	20.68 ± 2.25	18.07 ± 1.40*
HBC	39.55 ± 9.33	32.75 ± 7.10*

*Mean difference is significant at p<0.05

Table 5- Age related sex dimorphic indices in Kuri cattle.

Paramet.	Young		Middle-age		Aged	
(cm)	Male	Female	Male	Female	Male	Female
ISRSOF	6.24±0.37	5.74±0.24	7.10±0.36*	5.72±0.40	7.10±0.44	6.02±0.31
ISLSOF	6.32±0.40	5.86±0.31	7.06±0.40	5.84±0.43	7.20±0.58	6.12±0.28
MICW	10.26±0.23*	9.14±0.39	11.04±0.16*	10.42±0.12	11.32±0.53	10.40±0.24
LICL	23.78±1.11	22.42±1.25	26.96±0.61**	22.28±0.55	27.22±2.65	25.46±1.00
ITLW	19.06±0.81	17.0±0.56	21.38±0.42**	18.24±0.49	21.60±1.32	18.98±0.55
HBC	30.92±1.44	27.08±3.06	42.90±1.45*	33.10±2.50	44.84±5.32	38.08±2.12

** Mean difference is significant at p<0.01, *Mean difference is significant at p<0.05

length (IFSL) and width (IMCL, SOBW and ILCL) is higher in the male but not significant.

All the parameters obtained on the ventral aspect of the skull were not significant. The overall hard palate length (HPL), the perpendicular palatine (IPSL2), the Choana width at the pterygoid crest (ChL1) and the interincisive suture are higher in the female, but the palatine process of the maxilla is longer in the male. The Choana length (ChL), choana width at the pterygoid hamulus (ChL2), Incisivo-occipital length (IOL), condylobasal length and maximum zygomatic width (MZW1 and MZW2) values are higher in the male. Values of the width of hard palate at molar 1 and molar 3 (PWM1 and PWM3) are higher in the male, but at premolar 1 (PWP1) is higher in the female, though. The values of the incisive fissure lengths and widths (RIFL, LIFL, RIFW and LIFW) are bilaterally higher in the male (Table 2).

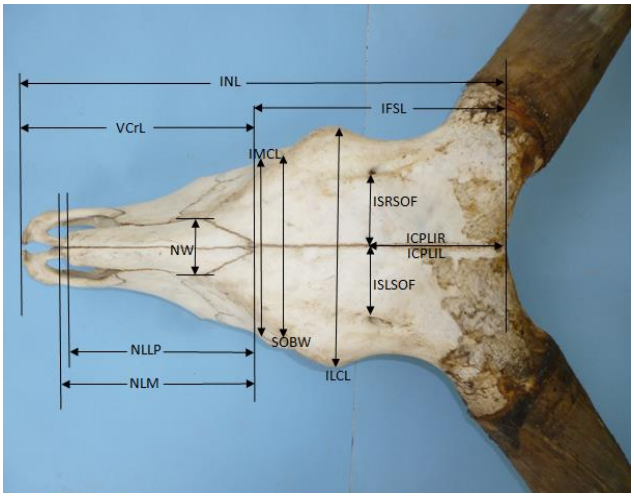

Figure 1:
Measurements of the Kuri cattle skull (dorsal view) showing Incisive – Nuchal length (INL), Supraorbital width (SOBW), Intermedial canthi length (IMCL), Interlateral canthi length (ILCL), Interfrontal suture length (IFSL), Interfrontal Suture to right supraorbital foramen (ISRSOF), Interfrontal Suture to left supraorbital foramen (ISLSOF), Intercornual protuberance to line of intersection of Right (ICPLIR), Intercornual protuberance to line of intersection of left (ICPLIL), Nasal length at midline (NLM), Nasal length at lateral prongs (NLLP), Nasal width (NW), and Viscerocranial length (VCrL).

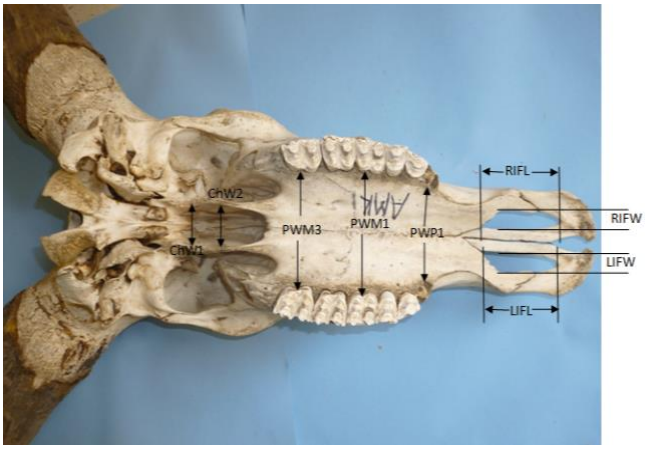

Figure 2:
Measurements of the Kuri cattle skull without mandible (ventral view) showing Left Incisive fissure length (LIFL), Right Incisive fissure length (RIFL), Left Incisive fissure width (LIFW), Right Incisive fissure width (RIFW), Palatine width at premolar 1 (PWP1), Palatine width at molar 1 (PWM1), Palatine width at molar 3 (PWM3), Choana width 1 (ChW1), and Choana width 2 (ChW2).

On the occipital and caudal aspects of the skull, only the maximum intercondylar (MICW) value showed statistical significance where the male recorded higher value, but all the other values were not significant. However, the intercornual protuberance to external occipital protuberance (IPEOP), Intercornual protuberance to foramen magnum (IPFM), interjugular process width (IJPW), interparacondylar width (IPCW), whole skull height (WSH1 and WSH2), and neurocranial volume (NCV) were also higher in the male. The foramen magnum height (FMH), foramen magnum width (FMW) and paracondylar process length (PCPL) values were higher in the female. Bilaterally, all the horn parameters taken on the rostral, caudal, lateral and medial aspects showed higher values in the male that were not significant, but the horn base circumference (HBC) value of the male was significantly higher.

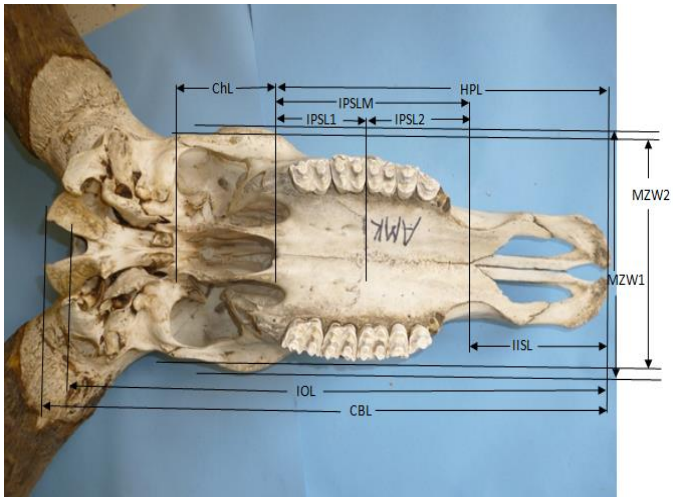

Figure 3:
Measurements of the Kuri cattle skull without mandible (ventral view) showing Hard palate length (HPL), Choana length (ChL), Maximum zygomatic width 1 (MZW1), Maximum zygomatic width 2 (MZW2), Interincisive suture length (IISL), Interpalatine suture length 1 (IPSL1), Interpalatine suture length 2 (IPSL2), Interpalatine suture length (IPSLM), Incisivo-occipital length (IOL), and Condylobasal length (CBL).

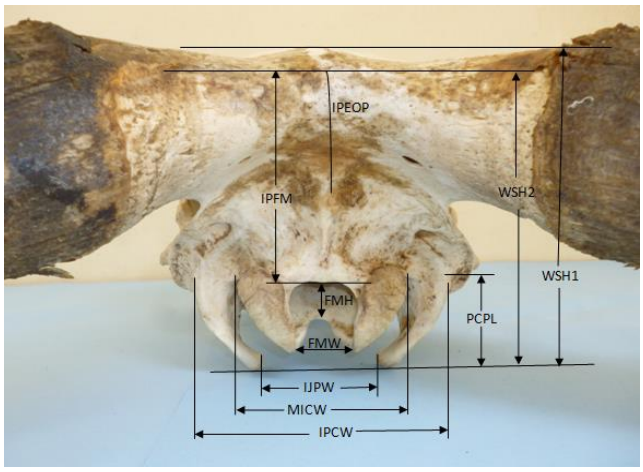

Figure 4:
Measurements of the Kuri cattle skull without mandible (Parieto-occipital view) showing Intercornual protuberance to external occipital protuberance (IPEOP), Intercornual Protuberance to Foramen magnum (IPFM), Foramen magnum height (FMH), Foramen magnum width (FMW), Maximum intercondylar width (MICW), Interjugular process width (IJPW), Paracondylar process length (PCPL), Interparacondylar width (IPCW), Whole skull height 1 (WSH1), and Whole skull height 2 (WSH2).

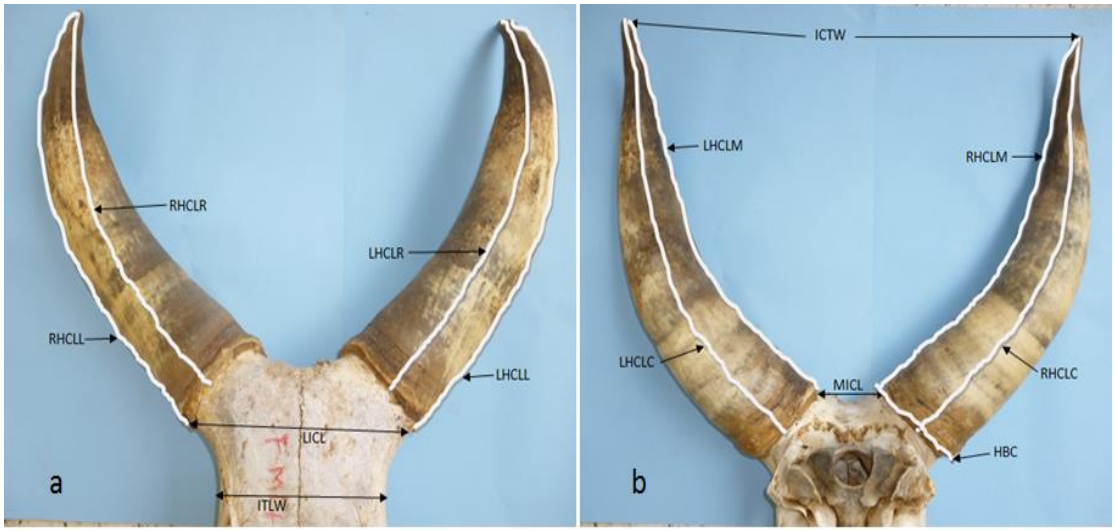

Figure 5: Measurements of the Kuri cattle skull (a. fronto-cornual and b. occipito-cornual view) showing a; Lateral intercornual length (LICL),), Right horn curvature length rostral (RHCLR), Left horn curvature length rostral (LHCLR), Right horn curvature length lateral (RHCLL), Left horn curvature length lateral (LHCLL), Intertemporal line width (ITLW). b; Medial intercornual length (MICL), Left horn curvature length caudal (LHCLC), Right horn curvature length caudal (RHCLC), Left horn curvature length medial (LHCLM), Right horn curvature length medial (RHCLM), Intercornual tip width (ICTW), and Horn base circumference (HBC).

DISCUSSION

To the best of our knowledge, this is the most comprehensive report in literature on the osteometry of skull of Kuri cattle. A total of 59 craniometric parameters were taken, and only 6 indices showed significant differences between the sexes. The result of this study revealed that the male Kuri cattle had significantly wider neurocranium (ISRSOF, ISLSOF, LICL and ITLW) with non-significant wider viscerocranial width (IMCL and NW). The differences observed on the neurocranial width could be sexual dimorphism. Sexual dimorphism was reported in neurocranial width by Kobrynczuk *et al.* (2008) in *"European bison"* (*Bison bonasus bonasus*), but was not significant in the Camel (Yahaya *et al.,* 2012b). The horn base circumference (HBC) value in this study is higher in the male, depicting that the HBC influenced the size of the lateral intercornual length (LICL) or vice versa.

All indices taken on the ventral aspect did not show any significant difference. The Choana length (ChL) is longer in the male, however, the hard palate length (HPL) value is higher in the female. This is further supported by higher value of the viscerocranial length (VCrL) of the female, indicating that the female Kuri cattle had longer viscerocranial length than the male. Insignificant higher palatal length values were reported in the female *Equus przewalskii f. cabalus* (Early Medieval horses) by Pasicka *et al.* (2012) and in goats (Olopade and Onwuka 2005). However, Yahaya *et al*. (2012b) reported insignificantly high palatal length for the male one humped camel. Conversely, the hard palate was insignificantly wider at the intermolar region in the male Kuri cattle, which also conforms to the work of Pasicka *et al*. (2012), and Yahaya *et al.* (2012b) as indicated above, but Olopade and Onwuka (2005) reported higher palatal width values in the female in West African Dwarf goat in Nigeria. The interfrontal suture (Neurocranium) length (IFSL) value is higher in the male; moreover, the overall skull length (INL) is higher in the male Kuri cattle as compared to the female. This is similar to what was reported in Early Medieval horses by Pasicka *et al.* (2012), in one humped camel (Yahaya *et al.* 2012b) and in lowland European bison (Kobrynczuk *et al.* 2008)

On the parieto-occipital aspect, only the base width between the lateral edges of the two occipital condyles i.e. maximum intercondylar width (MICW) showed significantly high value in the male Kuri cattle. This difference could be as a result thickness of the bases of the occipital condyle since the foramen magnum width value is higher in the female Kuri cattle. The foramen magnum height (FMH) and Paracondylar (jugular) process length (PCPL) were insignificantly higher in the female. Conversely, these values are higher in the young male one hump camel as compared to its female counterpart in Nigeria (Yahaya *et al.* 2012b). However, according to Samuel (2016) the foramen magnum height value is higher in the female, but wider foramen magnum width in the male *Procyonoides cancrivorous* (African giant Rat).

Out of the six indices that showed significant sex dimorphism (Table 5), only MICW showed sex dimorphism at two age groups; the young and middle-age groups. HBC and ISRSOF present sex dimorphism only at the middle-age group, while ISLSOF showed sexual dimorphism only on the cumulative value (n=15 for each sex) but not at any specific age group. ISFSOF and ISLSOF values are clinically important in locating the supraorbital foramen to track supraorbital

nerve in clinical procedures such as dehorning and trephination of frontal sinus. LICL and ITLW values showed significant sex dimorphism at $p<0.01$ in the middle-age group with the male having higher values than the female. The aged group did not indicate any parameter with significant difference between the sexes.

In conclusion, the middle-age Kuri cattle (3-5 years old) have the most sexually dimorphic osteometric landmarks in the skull with the male having higher values than the female. This data will be useful for anatomical, developmental, anthropological, forensic and clinical studies, and forms bases for comparison with other breeds of cattle.

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Comparative Neuroprotective Effect of *Celosia argentea Linn.* and Vitamin E on Mercury-induced Oxidative and Histological Parameters of Rat Brain

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Summary: Mercury contamination of our environment in Nigeria is increasing as mining activity increases. Its exposure causes human toxicological effects which include neurotoxicity through reactive oxygen species. This study investigated the ameliorative effects of the flavonoid-rich aqueous extract of *Celosia argentea* (AECA) and vitamin E (VitE) in the brain of rats treated with mercuric chloride ($HgCl_2$). Twenty-five adult male Wistar rats were randomized into five treatment groups (n=5). Group 1- control; Group 2- $HgCl_2$ (4 mg/kg); Group 3- AECA (400 mg/kg); Group 4- $HgCl_2$ (4 mg/kg) + AECA (400 mg/kg); Group 5- $HgCl_2$ (4 mg/kg) + VitE (500 mg/kg). All items were administered using an oral cannula daily for 14 days. Behavioural studies were carried out on the 16th day of experiment after which rats were euthanized. Thereafter, gross, haematological and biochemical parameters [malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)] were assessed. Mercuric chloride significantly ($p<0.05$) reduced body weight of rats, SOD activity and GSH level but increased MDA level, CAT activity and the number of degenerated neurons in the cerebral cortex relative to control group. Microscopically, $HgCl_2$ induced degeneration of cerebral cortical neurons and Purkinje neurons of the cerebellum. Treatment of $HgCl_2$ and AECA and VitE caused a reversal of these $HgCl_2$-induced alterations. The behavioural and haematological parameters were not significantly affected through the groups. The results suggest *Celosia argentea Linn* and vitamin E protected against mercury-induced gross, oxidative, cerebral and cerebellar damage. Both AECA and Vitamin E demonstrated neuroprotection in this experiment.

Keywords*:* Neuroprotection, *Celosia argentea*, Oxidative stress, Mercuric chloride, Cerebrum, Purkinje neuron.

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INTRODUCTION

Mercury is a heavy metal that can be found in the environment in three forms namely: elemental mercury or metallic mercury (Hg_0), inorganic mercury (mercuric chloride, $HgCl_2$), and organic mercury (methyl mercury, MeHg), the latter being the most common form of intoxication in humans. However, MeHg is gradually metabolized to inorganic mercury by intestinal microflora at a low rate per day (Bernhoft, 2012). Inorganic mercury has been used for many years in medications, teething powders, skin creams and germicidal solutions thus exposing humans to its toxicological effects (Goldman *et al*., 2001). Despite its low liposolubility, inorganic mercury can be detected in the brain, disrupting neuronal homeostasis (Clarkson and Magos, 2006) which is an evidence of its ability to accumulate in the body causing CNS damage (Smith *et al*., 1994). In Nigeria, mercury exposure and toxicity has become important as gold mining activity increases in Zamfara state, Niger state and other mining areas. Mercury exposure has been shown to stimulate the rate of reactive oxygen species (ROS) production leading to oxidative stress (Abdel Moneim, 2015). The free radical stimulation mechanism of neurotoxicity of mercury suggests that natural products with antioxidant components and free radical scavenging capability can ameliorate or protect the brain from the effects of mercury, hence the consideration of a plant part from *Celosia argentea.*

Celosia argentea is an important tropical leafy vegetable crop of high nutritional value (Aladesanwa *et al*., 2001) belonging to the Amaranthacea family. It is popular vegetable in Nigeria, where it is known as "soko yokoto", (Yoruba) meaning *"make husbands fat and happy"*, "eriamionu" (Igbo) meaning "*eat and smack your lips*" and "alayyaho daji" (Hausa). There could also be a red "soko" because it has red pigment on the leaves, which differentiates it from the green "soko" (Malomo *et al*., 2011). The presence of phenols and flavonoids which suggests antioxidant properties in *Celosia argentea L.* has been reported (Malomo *et al*., 2011, Verma and Demla, 2012 and Ramesh *et al.*, 2013). Its antioxidant properties suggest a possible

role in the search for substances that can reduce mercury toxicity.

Alpha-tocopherol (vitamin E), a fat-soluble vitamin has been demonstrated as a potent antioxidant and radical scavenger in chemical and biological systems, and protects the cell membrane from injury through its ability to prevent oxidation of unsaturated fatty acid (Cerecetto and Lopez, 2007). A previous study showed the potency of vitamin E in reducing lipid peroxidation by about 80% compared with 65%, for methanolic extract of *Vernonia amygdalina* in brains of rats exposed to oxidative stress via gamma irradiation (Owoeye et al., 2010).

The cerebellum and cerebral cortex were reported to be the targets of mercury intoxication (Xu et al. (2012), extensive damage to the hippocampal formation of rats has also been reported (Owoeye and Farombi, 2015). The Purkinje cells provides the output of the cerebellar cortex to the cerebellar nuclei (Zeeuw and Hoogland, 2015), thus important in regulation of motor coordination, equilibrium, muscle tone while the cerebral cortex is responsible for regulating cognition and primary sensory functions among other functions (Snell, 2006). The abundant lipid content and relative deficit in antioxidant systems compared to other tissues and high oxygen demand makes the brain susceptible and particularly vulnerable to damage ROS than do most other organs (Ebokaiwe et al., 2013). This susceptibility of the brain to neurotoxicants may affect its microanatomy and physiology in the absence of ameliorating factors.

In view of the scanty literature on the effect of AECA on mercury toxicity in the brain of rat, this study was carried out to investigate the possible protective effect of aqueous extract of *Celosia argentea* (AECA) in mercury-induced oxidative stress in the brain of adult male rats and then compare such effects with those of a standard antioxidant vitamin E. This study will thus answer the research question of whether the aqueous extract of *Celosia argentea* (AECA) can modify the effect of mercuric chloride on the brain of rat.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Celosia argentea Linn* was purchased from Bodija Market, Ibadan, Nigeria in the month of June, 2015 and was authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. A voucher specimen (FHI. 110229) was deposited at the herbarium of FRIN.

Extraction of the Aqueous Extract of *Celosia argentea L.*

The leaves were prepared using the method of Malomo *et al.* (2011). Briefly, the leaves were oven-dried at 40°C at a constant weight and the dried leaves were thereafter pulverized. Five hundred gramme of the powdered leaves was extracted in 5 L of distilled water at room temperature for 48 hours before been filtered. The filtrate was concentrated over a rotatory evaporator and dried to constant weight in an oven. This gave a yield 37.9% of aqueous extract of *Celosia argentea* and was given the acronym "AECA".

Preparation, dosage and administration of AECA

From a stock solution of 1 g AECA/ 10 mL of distilled water, the extract (AECA) was administered to the animals orally with the aid of an oral cannula at a dose of 400 mg/kg daily for fourteen (14) days. Dosage was based on the method of Malomo et al. (2011).

Preparation and administration of mercury chloride ($HgCl_2$)

Dry powder of Mercury Chloride ($HgCl_2$, 99% purity) manufactured by Loba Cheme PVT Ltd, Mumbia, 40005, India. From a stock solution of 500 mg of $HgCl_2$ to 20 mL of distilled water, $HgCl_2$ was administered to the animal at a dose of 4 mg/kg/day with the aid of an oral gavage for 14 days. Dosage was based on published method (Sheikh et al., 2013).

Preparation, dosage and administration of vitamin E

Each soft gelatin capsule containing 100 mg of DL-α-tocopheryl acetate as 100 mg vitamin E acetate (Sinopharm Xingsha Pharmaceuticals Co. LtD, China). Each 100 mg capsule was punctured with a new size 21G needle (Hypojet, Spain) attached to a new 1 mL hypothermic syringe, completely aspirated and then attached to a clean cannula through which each rat was administered orally the measured dose of 500 mg/kg body weight/daily (Owoeye et al., 2010).

Ethical approval

The research protocol was approved by the Animal Care and Use Research Ethics Committee (UI-ACUREC) of the University of Ibadan, Nigeria, with reference number UI-ACUREC/App/2014/003. The experiments were carried out at the Anatomy Department, while biochemical assays were carried out at the Drug Metabolism and Toxicology Unit, Department of Biochemistry and haematological studies were done at the Veterinary Pathology Laboratory of University of Ibadan. The animals received humane care in accordance with the principle of humane care and use of laboratory animals.

Experimental Animals

Adult male Wistar rats, weighing 150–240 g, aged about 3 months, were obtained from the breeding colony of the Department of Veterinary Physiology University of Ibadan, Nigeria. Five animals were kept in plastic cages having dimensions of 39 × 29 × 27 cm and soft wood shavings employed as bedding in the cages. They were housed in the College of Medicine Central Animal House in a light/dark cycle and had access to rat pellets (Ladokun Feeds, Mokola, Ibadan)

and water *ad libitum* where they were acclimatized for two weeks before randomization into experimental and control groups.

Experimental Design

Twenty five adult rats were after acclimatization divided into five groups of five animals each as detailed below:

Group 1: served as control, received distilled water only
Group 2: received $HgCl_2$ (4 mg/kg of $HgCl_2$)
Group 3: received 400 mg/kg of AECA extract
Group 4: received $HgCl_2$ (4 mg/kg of $HgCl_2$) + 400 mg/kg of AECA extract
Group 5: received $HgCl_2$ (4 mg/kg of $HgCl_2$) + 500 mg/kg of VitE.

AECA = Aqueous extract of *Celosia argentea L.*, $HgCl_2$ = Mercury chloride, VitE = Vitamin E.
All treatments were administered to the rats for 14 days orally by gavage.

Behavioural Tests

On experimental day 16, rats in each group were weighed and then subjected to behavioural studies namely **o**pen field test and forelimb grip strength test.

Open Field Test. The animals were placed for 5 min in an open-field arena. The apparatus, made of wood covered with impermeable formica, had a white floor of 100 × 100 cm (divided by black lines into 25 squares of 20 × 20 cm) and 40-cm high white walls. Each rat was placed at the center of the open field and was free to explore the unfamiliar arena; the total number of squares crossed and rearing was measured (Olopade *et al.*, 2012). The quadrant was considered crossed when the animal has four paws in the adjacent square. The test was carried out on day 15 of experiment.

Forelimb Grip Strength Test: This test involved the forepaws of the rats being placed on a horizontally suspended metal wire (measuring 2mm in diameter and 1m in length), placed one meter above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded. A maximum time of 2 minutes was given to each rat after which it was removed. The test reflects muscular strength in the animals (Olopade *et al.*, 2012).

Haematological Studies

On completion of behavioural studies on the 16th day of the experiment, blood for haematological parameters was obtained from the retro-ocular plexus of the animals using heparinized capillary tubes into Lithium heparin treated sample bottles for the determination of blood parameters namely: red blood cell count (RBCC), white blood cell count (WBCC), haemoglobin count (HB), packed cell volume count (PCV) and white cell differential cell count. These procedures were performed at the Veterinary Pathology Laboratory of the University of Ibadan, Nigeria.

Assessment of Oxidative Stress Parameters

The left hemisphere of the brain was used for biochemical assays. Malondialdehyde (MDA) level was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale (1990). The activity of Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (1972) and SOD activity was expressed as µ/mg protein. Catalase (CAT) was determined according to the method of Clairborne (1985) and enzyme activity was expressed as U/mg protein. The method of Beutler *et al.* (1963) was followed in estimating the level of reduced Glutathione (GSH) and was expressed in µmole/mg protein.

Histology

The animals were sacrificed by cervical dislocation on the 16th day of the experiment after final body weight measurement and blood samples were obtained. The whole brain was dissected and brain weights recorded. The full brain was divided into two halves using the method of Owoeye and Onwuka (2016); the right hemisphere was fixed in 10% neutral buffered formalin (10% NBF), dehydrated using a grade ethanol series and embedded in paraffin and then sectioned at 6 - 7µm thickness. Sections were stained with haematoxylin and eosin. The tissue sections were evaluated under light microscope (Olympus BX51, Tokyo, Japan) and photographed with a digital camera (100 Olympus, Olympus Optical Co. Ltd., Japan). The left hemisphere of the brain preserved for biochemical assays was rapidly rinsed, mopped with filter paper, weighed and kept in freshly prepared cold phosphate buffered solution (PBS) at pH=4 in the freezer till processed.
Histomorphometric analysis was done using computerized image analyzer (TSView CX image software file version 6.2.4.3 and Image motic 2000 (China). On the cerebral cortex sections, the number of non-viable pyramidal cells in the external pyramidal cell layer was counted per 5 high-power fields (x400).

Statistical Analysis

Data were analysed using one way ANOVA (Analysis of Variance) test, followed by Bonferroni's post-test analysis using the statistical software package Graphic Prism Version 5.04 (2010). The statistical significance was set at $p < 0.05$, for the null hypothesis being true by chance and the confidence interval at 95% level.

RESULTS

Body and brain weight changes

Mercuric chloride significantly ($p<0.05$) reduced the body of rats compared with control at the end of this

Table 1: Effect of mercuric chloride and *Celosia argentea* on body and brain weight of rats.

Groups	Initial BW (g)	Final BW (g)	Difference (g)	Brain Weight (g)	Relative brain weight (%)
CTRL	177.50±15.00	215.00±12.90	37.5±3.60	1.580±0.05	0.73±0.05
$HgCl_2$	220.00±21.60	212.50±17.07	-7.50±0.18♯	1.787±0.07	0.84±0.08
AECA	171.25±6.29	192.50±17.07	20.75±1.40*	1.632±0.04	0.85±0.06
$HgCl_2$+AECA	183.00±8.24	192.50±16.58	9.5±1.25⁎	1.672±0.15	0.87±0.11
$HgCl_2$+VitE	195.00±8.40	181.25±13.14	-13.75±0.05⁎	1.715±0.09	0.95±0.03

Values are expressed as mean ± SD of 5 rats. CTRL=Control, AECA=*Celosia argentea*, $HgCl_2$=Mercuric chloride, VitE = Vitamin E. BW=Brain weight. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

Table 2: Effect of mercuric chloride and *Celosia argentea* on behavioural parameters and forelimb grip of male Wistar rats.

Groups	Fore limb grip (s)	No. of grooms	No. of squares crossed	No. of rearing
CTRL	7.87±4.93	45.52±2.18	29.50±15.15	8.25±06.39
$HgCl_2$	8.25±2.59	42.75±2.59	30.75±24.91	13.25±09.17♯
AECA	5.75±1.19	44.75±1.19	32.75±17.65	6.50±00.57
$HgCl_2$+AECA	6.25±1.70	38.75±1.70	36.50±9.32	13.50±05.19
$HgCl_2$+VitE	4.62±0.47	75.25±0.47*	26.00±24.49	13.75±08.73

Values are expressed as mean ± SD of 5 rats. CTRL = Control, AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, VitE = Vitamin E. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

Table 3: Effect of mercuric chloride and *Celosia argentea* on red blood parameters.

Groups	PCV%	RBC (10_6/µL)	HB (g%)
CTRL	52.25±0.96	8.44±0.05	17.33±0.28
$HgCl_2$	54.75±1.71	8.56±0.06	18.28±0.40
AECA	50.00±2.94	8.29±0.48	16.60±0.83
$HgCl_2$+AECA	47.00±2.71	7.93±0.47	15.50±0.74
$HgCl_2$+VitE	50.50±0.58	8.46±0.07	16.65±0.13

Values are expressed as mean ± SD of 5 rats. CTRL = Control, AECA = *Celosia argentea*, $HgCl_2$ = Mercuric Chloride, VitE = Vitamin E.

Table 4: Effect of *Celosia argentea* and vitamin E on mercuric chloride-induced antioxidant status of rat brain.

Groups	MDA (µmol/mg.pr-)	SOD (Units/ mg.pr-)	CAT(U/mg.pr-)	GSH (µmole/ mg.pr-)
CTRL	5.002±0.60	0.799±0.29	19.09±0.91	1.037±0.09
$HgCl_2$	22.56±0.70♯	0.570±0.21	23.16±0.40♯	1.011±0.08
AECA	6.365±2.38	1.028±0.06♯	15.13±1.35♯	1.322±0.02♯
$HgCl_2$+AECA	7.900±1.70⁎	1.314±0.15*	25.23±1.18	1.352±0.05⁎
$HgCl_2$+VitE	8.111±0.56⁎	0.933±0.37*	23.21±0.98	1.183±0.04*

Values are expressed as mean ± SD of 5 rats. CTRL=Control, AECA=*Celosia argentea*, $HgCl_2$=Mercuric chloride, VitE = Vitamin E. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

experiment (Table 1) but co-treatment of $HgCl_2$ with AECA reversed this alteration relative to $HgCl_2$ group whereas co-treatment with Vitamin E did not. There were, however, no significant differences in the relative weight of rat's brain across the experimental groups as shown in Table 1.

Behavioural test assessments

Table 2 shows there was no significant difference between the control and other experimental groups in the number of squares crossed and forelimb grip strength. Rearing number was significantly ($p<0.05$) increased in the $HgCl_2$ alone group compared with the control. Only the $HgCl_2$ +VitE treatment elevated the number of grooms significantly ($p<0.05$) when compared with the control group**.**

Haematological parameters

Generally, there were no significant differences between the control and experimental groups as regards the red blood cell parameters (Tables 3) and the leukocytes (not displayed).

Biochemical analysis of antioxidant parameters

As shown in Table 4, $HgCl_2$ increased the MDA level significantly ($p<0.05$) when compared with control which co-treatment of $HgCl_2$ with AECA or VitE reduced relative to the $HgCl_2$ group. Additionally, $HgCl_2$ caused a reduction in the activity of SOD and level of GSH whereas it significantly elevated the activity of CAT compared with control. However, co-treatment of $HgCl_2$ with AECA or VitE reversed theses alterations relative to $HgCl_2$ group.

Table 5: Effect of mercuric chloride and *Celosia argentea* on viability of pyramidal cells of the external pyramidal layer of the cerebral cortex.

Groups	Mean number of non-viable pyramidal cells **/hpf**
CTRL	4.01±0.01
$HgCl_2$	185.00±7.70♯
AECA	5.01±0.30*
$HgCl_2$+AECA	39.00±0.60*
$HgCl_2$+VitE	25.00±0.56*

Values are expressed as mean ± SD of 5 rats. CTRL= Control, AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, VitE = Vitamin E, hpf = high power field. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

Histological examination of cerebral and cerebellar cortices

Figure 1C demonstrated the varying degrees of degeneration observed in cortical neurons elicited by $HgCl_2$ treatment when compared with control. The ameliorative effect of AECA and VitE relative to $HgCl_2$ group was demonstrated in Figures 1D and E. In Figure 2B, most of the Purkinje neurons of the cerebellum were devoid of nuclei material (karyorhexis) after treatment with $HgCl_2$ when compared with the control group. However, co-treatment with AECA and VitE demonstrated some protection relative to the $HgCl_2$ group (Fig. 2D and E).

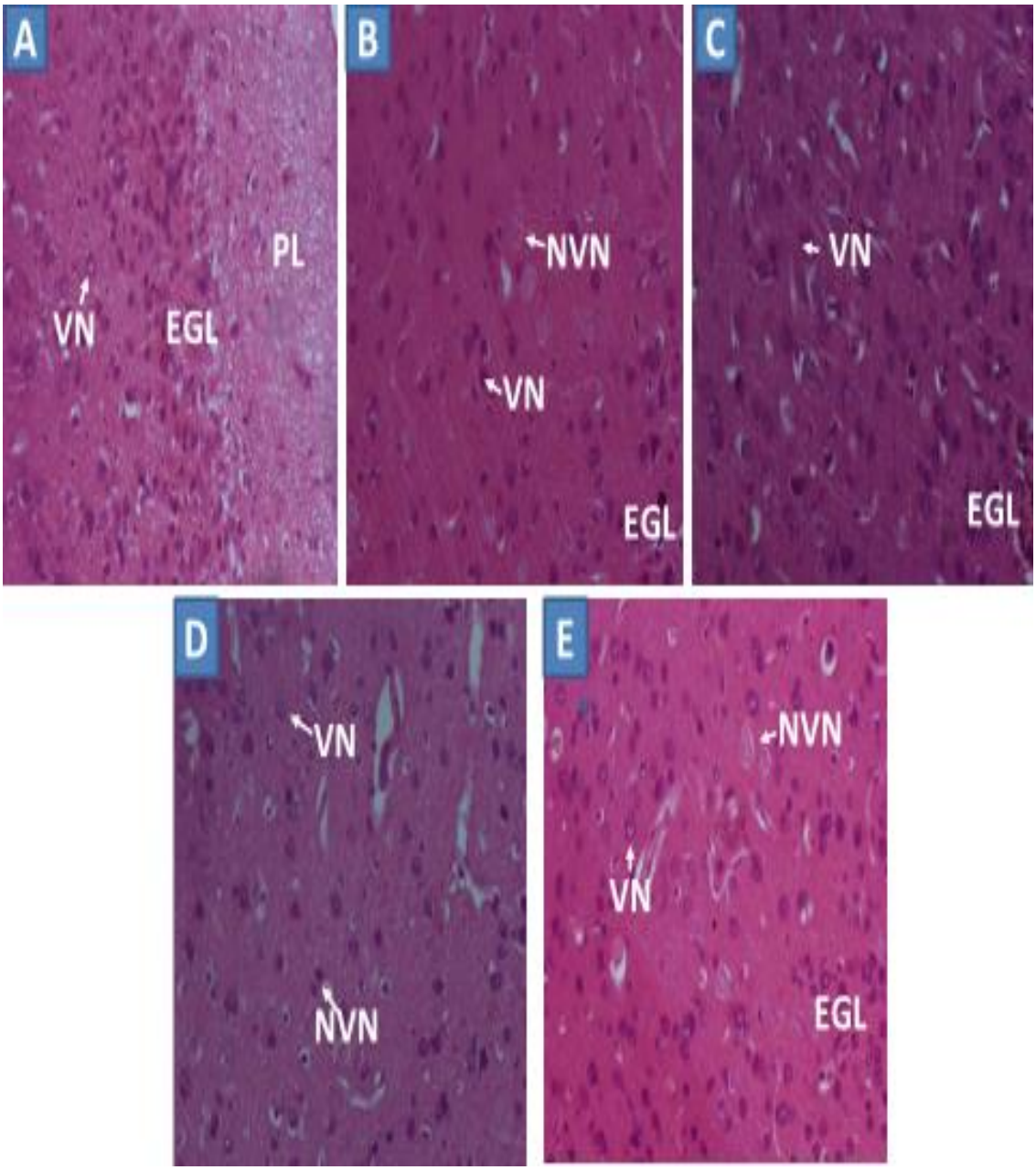

Fig. 1: **Representative photomicrograph of cerebral cortex.** (A) Control (B) $HgCl_2$ (C) AECA (D) $HgCl_2$+AECA (E) $HgCl_2$+VitE. PL = Plexiform layer, EGL = External granular layer, VN = Viable cortical neuron, NVN = non-viable cortical neuron. AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, VitE = Vitamin E. H&E x 400.

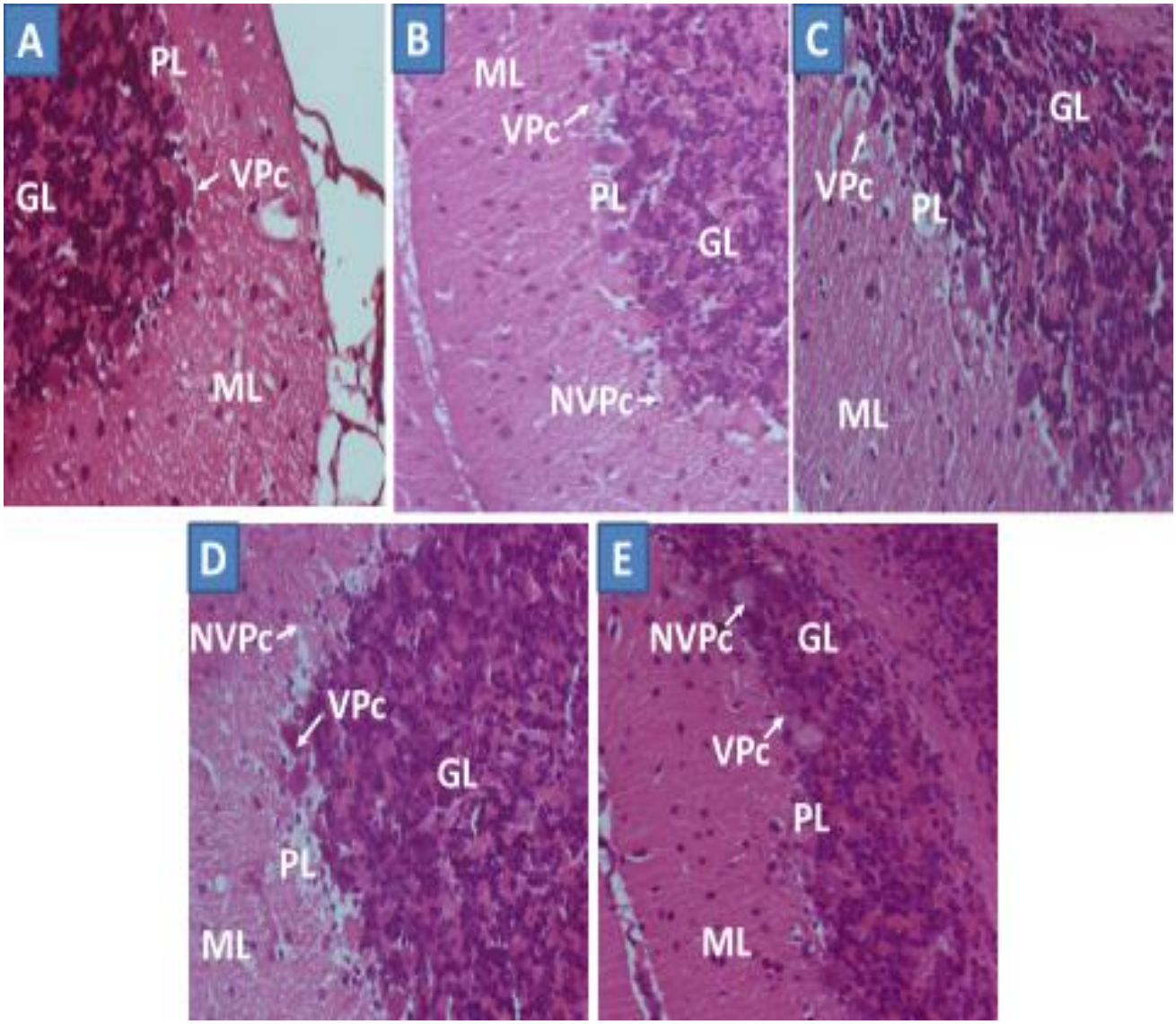

Fig. 2: **Representative photomicrograph of cerebellar cortex.** (A) Control (B) $HgCl_2$ (C) AECA (D) $HgCl_2$+AECA (E) $HgCl_2$+VitE. ML=Molecular layer, GL=Granular layer, PL= Purkinje layer, VPc=Viable Purkinje cell, NVPc=Non-Viable Purkinje cell. AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, Vit E = VitE. H&E x 400.

Histomorphometry evaluation of pyramidal neurons

Mercuric chloride increased the number of degenerated pyramidal neurons in the cerebral cortex significantly ($p<0.05$) when compared with the control (Table 5). This was reduced in the $HgCl_2$+AECA and $HgCl_2$+VitE treatment groups compared with the $HgCl_2$ thus demonstrating the ameliorative effect of AECA and VitE cotreatment with the toxicant as shown in Table 5.

DISCUSSION

In this present study, our data demonstrated the capability of the aqueous extract of *Celosia argentia* (AECA) and vitamin E to ameliorate the oxidative and histological alterations induced by mercuric chloride ($HgCl_2$) treatment of male rats.

Although body weight change serves as a sensitive indication of the general health status of an animal, gross changes in organ weight and weight coefficients (organ–body weight ratio) induced by chemical substances are a reliable marker of toxicity (Elias and Nelson, 2012). Therefore, the significant reduction of body weight in the $HgCl_2$-treated rats signifies its toxicity on the general wellbeing of the rats, whereas the lack of a significant increase in the absolute brain weight brain of rats across the groups might have indicated the absence of inflammation in the brain (Rossi et al, 2003). There was no significant alterations in the haematological parameters in agreement with previous studies (Owoeye and Farombi, 2015; Owoeye and Arinola, 2017).

Behavioural changes showed little effect of the different treatments on the motor abilities of the various groups although the results suggest some anxiety in the $HgCl_2$ +VitE treatment group as demonstrated in the increased number of grooming.

Our observation of increased lipid peroxidation demonstrated by increased thiobarbituric acid reactive

substances (TBARS) formation in rat brain treated with mercury alone $HgCl_2$ is supported by previous reports (Hussain *et al*., 1997; Ibegbu *et al.,* 2014). This was an indication of free radical generation induced by mercury toxicity as one of its mechanisms is overproduction of ROS (Abdel Moneim, 2015). The brain contains large amounts of polyunsaturated fatty acids and is particularly susceptible to free radical attack and, therefore, lipid peroxidation (Ebokaiwe, 2013).

The reduction of MDA in the $HgCl_2$+AECA group when compared with $HgCl_2$ group demonstrated the antioxidant capacity of *Celosia argentea Linn* extract which is in agreement with the report of Rukhsana *et al.* (2013). So also did the reduction of lipid peroxidation in the $HgCl_2$+VitE as indicated by lower levels of MDA when compared with $HgCl_2$ demonstrated the potency of the antioxidant capacity of vitamin E. While the decline in GSH levels in the $HgCl_2$ group is a reflection of the oxidative stress it induced (Vekaria, 2012), its elevation in the $HgCl_2$+AECA and $HgCl_2$+VitE groups relative to the $HgCl_2$ group demonstrated the potency of AECA and VitE to protect the brain against oxidative damage.

Superoxide dismutase is an important endogenous antioxidant that deals with superoxide radicals converting them to H_2O_2 (Chaudhary *et al*., 2003). Reports have shown that antioxidant enzyme activities might be reduced after Hg exposure *in vivo* (Vijayaprakash *et al*., 2013). However, the elevation of SOD in the $HgCl_2$+AECA and $HgCl_2$+VitE groups indicated the enhancement of the depressed activity of the enzyme by the antioxidant property of both AECA (Malomo et al., 2011) and VitE (Ulatowski et al., 2014). The elevation of the activity of CAT by $HgCl_2$ has the potential of increasing its ability to decompose H_2O_2 and convert it to water and diatomic oxygen. In AECA-treated rats there was an increase in the activity of the antioxidant enzyme SOD, and the level of GSH both of which play important roles in the detoxification of many environmental chemicals (Malomo et al., 2011). The decrease in the activity of SOD in $HgCl_2$-treated rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidant enzymes (Ansar, 2015).

With the exception of the vertical movement (rearing), mercuric chloride did not alter behavioural results suggesting that AECA and VitE did not significantly influence the behavioural parameters studied in rats with induced mercury-toxicity in this study.

Histology results clearly demonstrated the neurotoxicity of $HgCl_2$ in both cerebral and cerebellar cortices by the degenerative features observed in the cerebral cortical neurons and the karyolitic and karyorhexic Purkinje neurons of cerebellum, which agrees with previous findings (Ferraro et al., 2009; Uma et al., 2012; Owoeye and Farombi, 2015; Owoeye and Arinola, 2017). The implication of damage to frontal cerebral cortical neurons is the attendant alteration of cortical functions like decision making, control of movement, cognition among others (Tranel, 2005). On the other hand, damage to Purkinje neurons has the consequence of reducing the cerebellar output to the deep cerebellar nuclear from where the major cerebellar output emerges to target organs like the spinal cord, vestibular nuclei, brainstem and the nucleus ventrolateralis of thalamus (Afifi and Bergman, 2005). Being the focal neuron of the cerebellar cortex on which all afferent fibres ultimately converges; such a damage done by $HgCl_2$ might affect the cerebellar coordinating activity of voluntary movement, posture and balance, as well as the coordination of the saccadic and slow eye movements with neck movements (Affi and Bergman, 2005). The recovery of the neurons of the $HgCl_2$+AECA and $HgCl_2$+VitE groups when compared with the $HgCl_2$ group suggested that antioxidant capacity of both AECA and VitE ameliorated the toxicity of effects of $HgCl_2$ qualitatively and quantitatively as shown in the histomorphometry results. This implies the possible sparing of these animals from the aforementioned consequences of mercury damage (Owoeye and Farombi, 2015) in both the frontal cortex and the cerebellum. Vitamin E appears to be more potent than AECA in their ameliorative and antioxidant activity as the latter preserved more viable cells compared with the latter. The protective effects of AECA have been attributed to the presence of compounds like flavonoids and phenolics (Malomo *et al*., 2011).

From our data, mercuric chloride demonstrated neurotoxicity in this experiment as shown by gross, biochemical and histological alterations observed. The antioxidant activity of AECA and VitE demonstrated protection against the gross, oxidative stress and neuronal damage induced by mercury toxicity in the rats.

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In vivo Safety Evaluation of a Nigerian Polyherbal Mixture in Female Wistar Rats

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Summary: The present study evaluates the oral safety and oral toxicity reversibility of a Nigerian Polyherbal Mixture (*NPM*) in female Wistar rats. In this study, acute oral toxicity was conducted on 20 female Wistar rats using the limit dose test of Up-And-Down Procedure of the OECD Acute Oral Toxicity Testing 425 guidelines at 5000 mg/kg of *NPM*. Additionally, 40 female Wistar rats (120-150 g) were divided into 4 groups (n=10) and orally treated with 10ml/kg of distilled water, 82 mg/kg, 410 mg/kg and 2050 mg/kg of *NPM*, respectively, for 90 days. Five rats from each group were sacrificed while the remaining rats in each group were kept for another 14 days for oral toxicities reversibility test. Blood samples and vital organs were obtained for biochemical, hematological and histological changes. Results showed that acute oral toxicity testing of *NPM* caused no death in any of the three sequentially treated rats and its estimated LD_{50} value was greater than 5000 mg/kg. Chronic oral treatment with 82-2050 mg/kg *NPM* caused significant elevations in the serum urea and creatinine and full blood count parameters (except differential WBC counts). The elevated renal function parameters were corroborated by dose-related histological changes of renal tubular congestions. also caused profound thrombocytosis and histopathological changes of pulmonary interstitial widening and gastritis. In conclusion, *NPM* may not be considered safe for consumption on prolonged use and at a high dose due to its profound tendencies to cause pulmonary fibrosis, nephrotoxicity, gastritis and thrombo-embolism. However, all the biochemical and hematological but histopathological alterations induced by *NPM* were reversed 14 days after the treatment cessation.

Keywords: Oral toxicity testing, Renal and hepatic function, Histopathological assessment, Reversibility test, Nigerian Polyherbal Mixture

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INTRODUCTION

The use of herbal remedies has been widely embraced in many developed countries with Complementary and Alternative Medicine (CAM) now becoming main stream in the United Kingdom and the rest of Europe as well as in North America and Australia (Calapai, 2008; Braun *et al.*, 2010; Anquez-Traxler, 2011). Herbal Medicine is very popular and widely used in the developing as well where they offer a wide range of available and affordable alternatives to conventional drugs. In Africa, for example, up to 80% of the population depends either partly or wholly on CAM according to World Health Organization estimate (World Health Organization 2005a; 2005b). Similarly, the new health agenda in Africa and indeed Nigeria focuses on the institutionalization of traditional medicine in parallel with Orthodox Medicine into natural Health Care Scheme in order to move the health agenda forward since effective healthcare in Africa cannot be achieved through Orthodox Medicine alone unless complemented with CAM (Elujoba *et al.*, 2005).

In recent years, issues relating to increasing use of herbal products in developed countries, dependence of many people living in developing countries on plants as a major source of medicines coupled with absence or weak regulation of herbal medicines in most countries and the occurrence of high-profile safety concerns, have increased awareness of the need to monitor safety and deepen understanding of possible harmful as well as potential benefits associated with the use of herbal medicines (Rodrigues and Barnes, 2013; Ekor, 2013). Similarly, in the Nigerian health sector, one of the main concerns is the indiscriminate use of drugs including packaged polyherbal mixtures popularly sold as "bitters". Many brands of the "bitters" which have flooded the Nigerian markets and

enjoy high patronage by their consumers include *Yoyo, Alomo, Orijin, Agbo, Action, Washing and Setting, Baby-Oku, Skelewu, Man-Power, Swedish, Goko Cleanser, Ruzu, Kerewa, etc*. These "bitters" are much sought after for their acclaimed health benefits which include amongst others energizer, improved mental alertness, sexual enhancement, blood cleansing/purification, pain relief, *etc*. For example, the polyphenol contents, *in vitro* antioxidant capacity and membrane stabilizing potential of some of these herbal remedies (namely Fijk, Osomo, Alomo and Oroki) were reported (Adeyemi and Owoseni, 2015). However, there are increasing health concerns which have been reported to be associated with the consumption of some of the existing supposed "health-promoting bitters" which include chronic kidney disease (Vivekenand, 2010; Zhang *et al.*, 2015), chronic liver disease (Abdulmajid and Sergi, 2013; Amadi and Orisakwe, 2018), pancreatic and heart diseases and sudden death (Farah *et al.*, 2000; Ernst, 2002; Ekor *et al.*, 2010). Subacute animal studies of Nigerian polyherbal remedies ('*Agyanom* mixture', '*Bolex* bitters' and '*Remedia* mixture') showed that the herbal mixtures were associated with mild to severe tubular necrosis indicating the mixtures have adverse effects on the kidney and they might not be safe for human consumption (Akande *et al.*, 2010a). Similarly, subacute animal studies with 1206.5 mg/kg/day and 804.3 mg/kg/day of the Nigerian "Yoyo bitters" for 28 days was associated with organ (liver and kidney) toxicities marked by lipid peroxidation (Adeyemi *et al.*, 2012) while its recommended doses precipitated immunomodulatory activities and hemolysis in Wistar rats (Oyewo *et al.*, 2013).

Despite the wide availability and application of polyherbal remedies/mixtures to promoting human health in Nigeria and indeed other African as well as in some Western countries, there is a dearth of scientific validation of the folkloric therapeutic efficacy as well as the scientific evaluation of their safety profile (Zhang *et al.*, 2015). Therefore, the current study was designed to evaluate both the oral toxicity and reversibility profile of *NPM* in the young adult female Wistar rats, which is strongly in line with the World Health Organization set goals on determining the safety profile of medicinal plants before it can become acceptable for human use.

MATERIALS AND METHODS

Sourcing and preparation of NPM: The herbal mixture used for this study is Oroki Herbal Mixture® produced by NURD Industrial and Commercial Company. It was purchased from the Company's Head Office at No. 4 Ifelodun Street, Off Agbado Adetola Bus-Stop, Ijaye, Lagos State, Nigeria. The leaflet described its constituents as *Sorghum bicolor* (5%), *Khaya grandifoliola* bark (10%), *Cassia sieberiana* root (3%), *Staudtia stipitata* root (3%), *Alstonia congensis* bark (5%), *Ocinum basillicum* leaves (7%), *Mangifera indica* leaves (7%), *Cyathula prostrata* leaves (7%), *Securidaca longependunculata* root (5%) and *Saccharum officinarum* stem (5%).

Five liters of *NPM* was concentrated to complete dryness in an aerated oven preset at 40℃. The solid residue left behind was scrapped, weighed on a Mettler weighing balance and kept in air- and water-proof container before it was stored in a refrigerator at -4℃. The percentage yield was 56.94 ± 8.4%.

Preliminary qualitative phytochemical analysis of NPM: Preliminary qualitative phytochemical analysis to confirm the presence or absence of secondary metabolites such as flavonoids, alkaloids, saponin, tannin, phlobatinnins, terpenoids, glycosides and anthraquinones in *NPM* were conducted using standard procedure as described by Sofowora (1993) and adopted by Adeneye *et al.* (2006).

Experimental animals: Sixty healthy and nulliparous female Wistar Albino female rats (120- 150 g) were obtained from Bayo's Animal Farm, Sango-Otta, Ogun State, after an ethical approval for the study was obtained. The rats were housed in the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State under controlled conditions with a 12 hour light/12 hour dark schedule and fed with commercially available rat pelleted diet (Animal Care Feeds, Ibadan, Oyo State) and water *ad libitum* throughout the period of the experiment. The rats were acclimatized for 14 days. Thereafter, twenty rats were randomly selected from the rat population for the acute oral toxicity studies using the Up-and-Down Procedure of the OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme- AOT425 StatPgm, version 1.0 as described by Adeneye *et al.* (2006).

The remaining forty rats were randomly divided into 4 groups of 10 rats such that the weight differences within and between groups do not exceed ±20% of the average body weight of the rat population. The rats which were housed in standard metallic cages were divided into four major groups and two sub-groups. The cage beddings and water bottles were cleaned on a daily basis and the experimental animals were handled in accordance with Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993) and international guidelines on the Handling and Care of Experimental Animals (United States National Institutes for Health, 1985). The whole experiment lasted for 104 days (90 days for subchronic toxicity testing and 14 days for oral toxicity reversibility testing).

Acute oral toxicity study of NPM using limit dose test of Up and Down Procedure in Wistar rats: The Acute oral toxicity study was conducted using the limit dose test of up and down procedure according to

OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425 StatPgm, version 1.0 as adopted by Adeneye *et al.* (2006), at a limit dose of 5000 mg/kg body weight/oral route and default of Sigma at 0.5. A total of 3 female young adult Wistar rats were systemically selected out of a population of 10 Wistar rats (8-12 weeks old) by systematic randomization techniques. The population sample was selected such that the weight differences do not exceed ±10% of the mean initial weight of the sample population. The rats were fasted of rat feed overnight prior to dosing on each occasion. A rat was picked at a time, weighed and dosed with equivalent 5000 mg/kg body weight of the *NPM* extract. After the extract administration, each rat was observed for the first 5 minutes after oral administration for signs of possible regurgitation and then kept in a cage for observation. Each was watched for every 15 min in the first 4 hour after dosing, then every 30 minutes for the successive 6 hours and then daily for the successive 38 hours for the short-term outcome and the remaining 12 days for the long-term possible lethal outcome.

Behavioral manifestations of acute oral toxicity were also observed such as restlessness, hyperactivity, dullness and general morphological changes. All observations were systematically recorded with individual records being maintained for each rat.

Subchronic oral toxicity studies of NPM in Wistar rats: The young adult nulliparous female Wistar rats were randomly divided into four major groups consisting of twelve rats each such that the weight differences within and between groups do not exceed ±20% of the average body weight and were all orally treated on daily basis for 90 days. Graded oral doses {82 mg/kg/day (sub-therapeutic), 410 mg/kg/day (therapeutic) and 2025 mg/kg/day (supra-therapeutic)} of the *NPM* extract administered to the Wistar rats for a period of 90 days were determined from the preliminary earlier conducted. The rats were randomly divided and allotted to Groups I-IV consisting of ten female Wistar rats per group. The allotment was done such that the average body weight within groups and between groups does not exceed 20% of each other. Group by group oral treatments of rats are as follows:

Group I: 10 ml/kg/day of distilled water
Group II: 82 mg/kg/day of *NPM* extract dissolved in distilled water
Group III: 410 mg/kg/day of *NPM* extract dissolved in distilled water
Group IV: 2025 mg/kg/day of *NPM* extract dissolved in distilled water

Measurement of body weight of treated rats: The weights of all the rats were taken using electronic Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland) on days 1, 30, 60, and 90 respectively.

Animal euthanasia and blood sample collection: A day prior to termination of the first phase of the experiment on day 90, the rats were fasted of feed except for potable drinking water which was still freely available. The rats were humanely sacrificed under deep inhaled diethyl ether anesthesia. Blood samples were collected directly from the rat heart chamber with a 21 G needle mounted on a 5 ml syringe plunger (Unique Pharmaceuticals, Sango Otta, Ogun State, Nigeria). 5 ml of blood sample for full blood count and biochemical (liver and renal functions) was collected into 10 ml capacity EDTA-treated blood sample bottles. The blood sample for the biochemical analysis was centrifuged with Uniscope Laboratory Centrifuge (Model SM 112, Surgifriend Medicals, England) at 3500 rpm for 10 minutes to separate the plasma from the blood cells. The plasma were carefully separated into new, well labeled, corresponding plain sample bottles at room temperature 23-26 ºC. The plasma obtained were used to analyze for the possible toxic effect of the *NPM* on the liver (using liver function test parameters such as ALT, AST, ALP, TP, ALB, TC and HDL) and kidney (renal function parameters such as electrolytes-sodium, potassium, calcium, chloride; urea and creatinine).

Measurement of organ weight and calculation of relative organ weight of rats: The rats were dissected and vital organs such as the lung, spleen, stomach, heart, liver, and kidneys were identified and dissected out *en bloc*. The organs were then rinsed of blood and weighed with electronic Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight of each organ relative to the 100 g of body weight of the rats from which the organ was harvested was calculated as: {Organ weight (g) ÷ body weight of rat (g) x100}

Determination of plasma renal function parameters: Plasma creatinine and blood urea were assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by standard quantitative methods (Peake and Whiting, 2006; Salazar, 2014). Plasma levels of sodium, potassium, chloride, calcium, bicarbonate and phosphate were determined using the ISE 6000 BYY SFRI spectrophotometer using the procedure earlier described by Adeneye *et al.* (2006).

Determination of plasma liver function parameters: Samples of the clear plasma obtained for were assayed for the following liver function parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, triglyceride, total cholesterol and cholesterol fractions [high density lipoprotein

cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein cholesterol (VLDL-c)], total (TB) and conjugated bilirubin (CB). Serum ALT, AST and ALP were measured using the enzyme kinetic method described by Peake *et al.* (1988) and Huang *et al.* (2006). Other biochemical determinations include triglyceride, total cholesterol and cholesterol fractions using method of Fossati and Principe (1982). The total protein was estimated by Biuret method (Okutucu *et al.*, 2007) while that of albumin was determined by the method described by Rees *et al.* (2012). The total bilirubin and the conjugated bilirubin were determined by Westwood method (1982).

Hematological Assays: Blood samples were collected directly from the heart chamber from anaesthetized rats with 12 G needle mounted on a 5 ml syringe plunger (Unique Pharmaceuticals, Sango-Otta, Ogun State, Nigeria) and collected into EDTA-treated bottles for full blood count on Automated Haematology System (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan). Full blood parameters measured include red cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), and differential neutrophils (%Neut.), differential lymphocytes (%lymph) and differential granulocytes (%Gran.).

Histopathological studies: After the dissected vital organs were rinsed and weighed, the organs were preserved in 10% formo-saline before they were completely dehydrated in absolute (100%) ethanol. The organs were then embedded in routine paraffin blocks. From the embedded paraffin blocks, 4-5 μm thick sections of each tissue was prepared and stained with haematoxylin-eosin. These were examined under a photomicroscope (Model N-400ME, CEL-TECH Diagnostics, Hamburg, Germany) connected with a host computer. Sections were illuminated with white light from a 12V halogen lamp (100 W) after filtering with a 520nm monochromatic filter (Thanabhorn et al., 2006). The prepared slides were examined for possible associated histological lesions.

Oral toxicity reversibility test of NPM: At the end of the chronic oral toxicity study period, all the animals are sacrificed humanely under anaesthesia with the exception of six randomly selected rats from each of the treatment and control groups. These were left untreated with the *NPM* extract but had free access to water and feed for 14 days. The rats were then fasted overnight and on the 15th day, all the remaining rats were sacrificed and had their blood samples collected for biochemical, haematological and histopathological assessment as described for chronic toxicity study. The reversibility study is aimed at evaluating if the biochemical, haematological and histopathological alterations induced in the course of the chronic oral toxicity study would become reversible with withdrawal of the oral extract treatment or not after 14 days (Adeneye *et al.*, 2010). Autopsy is performed on all animals and vital organs weighed and examined for gross and histological lesions (Ibrahim *et al.*, 2018).

Data Analysis: All data were expressed as mean ± S.D. for body weight and relative organ weights while data for biochemical and hematological assays were expressed as mean ± S.E.M. Significant differences among the group were determined by One-way analysis of variance (ANOVA) and *post hoc* test determined by Newman-Keuls test using GraphPad Prism 5 software. Results were considered to be significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

RESULTS

Percentage Yield of NPM extract: Complete drying of *NPM* resulted in dark brown, sweet-smelling and sticky solid residue with an average yield of 56.94 ± 8.4%.

Preliminary Qualitative Phytochemical Analysis of NPM: Preliminary phytochemical analysis of *NPM* showed the presence of phenols, flavonoid, saponin, terpenoids, cardiac glycosides, steroid glycosides and anthraquinones while tannin, alkaloids and phlobatannin were absent.

Acute Oral Toxicity Studies of NPM Using the Limit Dose Test of Up and Down Procedure: Single oral treatment of rats with 5000 mg/kg body weight of *NPM* produced no lethality within the short- and long-term outcome of the limit dose test of Up-and-Down Procedure (Table 1). However, the resulting behavioral toxicity signs observed included irritation, bilateral narrowing of the eyelids and abnormal posture (which was characterized by tugging of the head in-between the hind-limbs) and feed refusal. However, the software-generated LD_{50} value calculated to be greater than 5000 mg/kg body weight/oral route.

Table 1.
Sequence and Results of Limit Dose test of Up and Down Procedure of Acute Oral Toxicity of *NPM* in treated female nulliparous Wistar rats

Test sequence	Animal ID	Dose (mg/kg)	Short-term result (48 h)	Long-term result (12 days)
1	01	5000	Survival	Survival
2	02	5000	Survival	Survival
3	03	5000	Survival	Survival

Table 2.
Effect of subchronic oral treatments with 82-2050 mg/kg of *NPM* on the average body weight (bwt) of treated rats on 1st, 30th, 60th and 90th day of treatment

Group	Day 1	Day 30	Day 60	Day 90
I	123.2 ± 9.1	135.0 ± 9.1	151.4 ± 7.2	172.1 ± 3.38
II	105.6 ± 17.0	135.0 ± 19.0	139.00 ± 18.3	167.5 ± 7.1
III	127.1 ± 24.2	118.4 ± 11.4	131.1 ± 11.3	170.9 ± 12.5
IV	125.8 ± 10.3	131.0 ± 10.7	149.2 ± 9.4	187.7 ± 22.8

Results are expressed as mean ± SD. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*

Table 3.
Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on relative weights of liver (RLW), kidney (RKW), lung (LGW), spleen (RSW), heart (RHW) and stomach (RSTW) in treated rats for 90 days

Organ	Groups			
	I	II	III	IV
RLW	4.58 ± 0.95	4.03 ± 0.23	4.01 ± 0.35	4.21 ± 0.87
RKW	1.13 ± 0.06	0.80 ± 0.06	0.80 ± 0.09	0.74 ± 0.17
LGW	1.93 ± 0.71	1.18 ± 0.10a	1.16 ± 0.10a-	0.91 ± 0.16b-
RSW	0.68 ± 0.47	0.53 ± 0.14	0.48 ± 0.09	0.42 ± 0.13
RHW	0.45 ± 0.08	0.43 ± 0.08	0.47 ± 0.06	0.38 ± 0.13
RSTW	2.95 ± 0.40	1.70 ± 0.37b	1.37 ± 0.24c	2.60 ± 0.80a

Results are expressed as mean ± S.D. a-, b- and c- represent significant reductions at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to control (Group I) values. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*

Table 4.
Effect of a 14-days oral toxicity reversibility test of 82-2050 mg/kg/day of *NPM* on relative weights of liver (RLW), kidney (RKW), lung (LGW), spleen (RSW), heart (RHW) and stomach (RSTW) in treated rats for 90 days

Organ	Groups			
	I	II	III	IV
RLW	3.64 ± 0.33	4.70 ± 0.68	3.99 ± 0.28	4.37 ± 1.18
RKW	1.18 ± 0.18	0.96 ± 0.04	1.00 ± 0.04	0.95 ± 0.08
LGW	1.12 ± 0.06	1.35 ± 0.04	1.25 ± 0.08	0.94 ± 0.08
RSW	0.76 ± 0.30	0.66 ± 0.11	0.60 ± 0.09	0.59 ± 0.17
RHW	0.73 ± 0.27	0.71 ± 0.16	0.75 ± 0.06	0.52 ± 0.05
RSTW	2.56 ± 0.79	3.91 ± 0.72	5.04 ± 0.34	3.39 ± 0.97

Results are expressed as mean ± S.D. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*

Effect of subchronic oral treatment with 82-2050 mg/kg/day of NPM on the average body weights (g) in treated rats: Single, daily oral treatment of rats with 82-2050 mg/kg of *NPM* for 90 days did not result in any significant ($p>0.05$) changes in the average body weight in any of the treatment groups on days 1, 30, 60 and 90 of the treatment when compared to that of the control group (Table 2).

Effect of subchronic oral treatment with 82-2050 mg/kg/day of NPM on the relative weights of liver (RLW), kidney (RKW), lung (LGW), spleen (RSW), heart (RHW) and stomach (STW) of treated rats: Single, daily oral treatment of rats with 82-2050 mg/kg of *NPM* for 90 days did not result in any significant ($p>0.05$) changes in the relative weights of liver, kidney, spleen and heart but caused significant dose-dependent reductions ($p<0.05$, $p<0.01$) in the relative lung weight and non-dose dependent significant reductions ($p<0.05$, $p<0.01$, $p<0.001$) in the relative stomach weight when compared to the control (Group I) values (Table 3).

Effect of oral toxicity reversibility of NPM on the relative weights of liver (RLW), kidney (RKW), lung (LGW), spleen (RSW), heart (RHW) and stomach (STW) of treated rats: Table 4 showed effect of oral toxicity reversibility of *NPM* on the relative weights of liver, kidney, lung, spleen, heart and stomach of rats after 14 days of ceasing the oral administration of *NPM*.

Effect of sub-chronic oral treatment with 82-2050 mg/kg/day of NPM on the plasma liver enzymes (ALT, AST and ALP), albumin (ALB) and total protein (TPR) of treated rats: Single, daily oral treatment of rats with 82-2050 mg/kg of *NPM* for 90 days did not result in any significant ($p>0.05$) alterations in the plasma levels of liver enzymes (ALT, AST and ALP), albumin and total

Table 5.
Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on the plasma liver enzymes (ALT, AST and ALP), albumin (ALB) and total protein (TPR) of treated rats

Groups	ALP (U/l)	ALT (U/l)	AST (U/l)	ALB (g/l)	TPR (g/l)
I	80.47±7.54	46.67±6.18	170.50±16.50	45.87±01.00	75.30±01.15
II	107.70±11.35	30.27±3.21	167.50±17.27	42.45±01.16	71.72±04.05
III	83.97 ±7.26	49.45±7.63	148.60±08.72	43.63±01.42	76.77±02.05
IV	92.23±8.86	37.60±5.51	182.00±34.43	43.77±01.66	76.22±02.54

Result expressed as mean ± SEM. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of NPM; III= 410 mg/kg/day of NPM; IV= 2050 mg/kg/day of NPM

Table 6.
Effect of 14-days oral toxicity reversibility test of 82-2050 mg/kg/day of *NPM* on the plasma liver enzymes (ALT, AST and ALP), albumin (ALB) and total protein (TPR) of treated rats

Groups	ALP (U/l)	ALT (U/l)	AST (U/l)	ALB (g/l)	TPR (g/l)
I	79.28±07.76	18.33±00.82	32.49±02.03	43.80±00.80	80.30±00.80
II	91.60±06.83	17.86±00.69	32.90±01.98	44.00±01.40	81.30±01.80
III	93.10 ±06.50	18.50±01.23	31.60±01.48	42.00±01.80	83.80±01.50
IV	86.55±06.69	18.17±01.46	31.91±01.22	41.30±00.50	85.30±00.70

Result expressed as mean ± SEM. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of NPM; III= 410 mg/kg/day of NPM; IV= 2050 mg/kg/day of NPM

Table 7.
Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on the plasma total cholesterol (TC), triglyceride (TG) and total bilirubin (TB) of treated rats

Treatment Groups	total cholesterol (mg/dl)	triglycerides (mg/dl)	total bilirubin (mg/dl)
I	72.59 ± 01.69	97.85 ± 07.32	00.67 ± 00.08
II	70.97 ± 03.77	87.20 ± 04.81	00.90 ± 00.26
III	67.55 ± 01.55	64.07 ± 03.99$^{\#}$	00.67 ± 00.10
IV	59.64 ± 01.17$^{\#}$	49.38 ± 02.00$^{\$}$	00.80 ± 00.90

Result expressed as mean ± SEM. # and $ represent significant decreases at p<0.001 and p<0.0001, respectively, when compared to the control (Group I) values. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of NPM; III= 410 mg/kg/day of NPM; IV= 2050 mg/kg/day of NPM

Table 8.
Effect of 14-days oral toxicity reversibility test of 82-2050 mg/kg/day of *NPM* on the plasma total cholesterol (TC), triglyceride (TG) and total bilirubin (TB) of treated rats

Treatment Groups	total cholesterol (mg/dl)	triglycerides (mg/dl)	total bilirubin (mg/dl)
I	91.58 ± 03.17	117.20 ± 14.87	00.54 ± 00.04
II	89.72 ± 03.45	90.17 ± 04.90	00.50 ± 00.05
III	87.21 ± 02.23	93.34 ± 05.64	00.55 ± 00.08
IV	97.11 ± 08.32	110.70 ± 10.83	00.53 ± 00.20

Result expressed as mean ± SEM. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of NPM; III= 410 mg/kg/day of NPM; IV= 2050 mg/kg/day of NPM

protein on the 90th day of the treatment when compared to the control (Group I) values (Table 5).

Effect of 14-days oral toxicity reversibility test of NPM on the plasma liver enzymes (ALT, AST and ALP), albumin (ALB) and total protein (TPR) of treated rats: Similar non-significant ($p>0.05$) alterations in the plasma liver enzymes, albumin and total protein levels were also seen in the 14-days oral reversibility test with *NPM* (Table 6)

Effect of subchronic oral treatment with 82-2050 mg/kg/day of NPM on the plasma liver total cholesterol (TC), triglyceride (TG) and total bilirubin (TB) of treated rats: Single, daily oral treatment of rats with 82-2050 mg/kg of *NPM* for 90 days caused significant decreases ($p<0.001$, $p<0.0001$) in the plasma total cholesterol and triglyceride levels when compared to the control (Group I) values (Table 7). However, oral treatment with *NPM* for 90 days did not cause any significant ($p>0.05$) alterations in the plasma total bilirubin levels in the treated rats when compared to control values (Table 7).

Effect of 14-days oral toxicity reversibility test of NPM on the plasma liver total cholesterol (TC), triglyceride (TG) and total bilirubin (TB) of treated rats: Withdrawal of oral treatment with 82-2050 mg/kg/day of *NPM* for 14 days resulted in the reversal of the earlier significant reductions ($p<0.001$ and $p<0.0001$) in the plasma total cholesterol and triglyceride with non-significant alteration ($p>0.05$) in the plasma albumin levels (Table 8).

Table 9.
Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on the plasma electrolytes (Na_{+}, K_{+}, Cl_{-} and HCO_{3-}) of treated rats

Groups	Na^{+}(mmol/l)	K^{+} (mmol/l)	Cl^{-}(mmol/l)	HCO_3^{-}(mmol/l)
I	145.80 ± 04.55	06.27 ± 00.17	95.90 ± 01.77	10.60 ± 02.65
II	147.10 ± 02.48	06.87 ± 00.22	94.60 ± 01.55	10.90 ± 00.95
III	137.90 ± 02.73	08.41 ± 00.15	93.63 ± 00.97	11.23 ± 00.21
IV	122.50 ± 02.67^{f-}	09.65 ± 00.11^{c+}	92.20 ± 00.94^{d-}	16.30 ± 00.86

Result expressed as mean ± SEM. $c+$ represents a significant increase at $p<0.001$ while $d-$ and $f-$ represent significant decreases at $p<0.05$ and $p<0.0001$, respectively, when compared to the control (Group I) values. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*

Table 10.
Effect of 14-days oral toxicity reversibility test of 82-2050 mg/kg/day of *NPM* on the plasma electrolytes (Na_{+}, K_{+}, Cl_{-} and HCO_{3-}) of treated rats

Groups	Na^{+}(mmol/l)	K^{+} (mmol/l)	Cl^{-}(mmol/l)	HCO_3^{-}(mmol/l)
I	139.40 ± 05.48	06.93 ± 00.87	99.73 ± 03.36	10.00 ± 00.35
II	140.00 ± 02.01	06.18 ± 00.86	96.98 ± 01.18	12.78 ± 01.09
III	150.20 ± 02.11	05.66 ± 00.69	96.99 ± 02.01	10.43 ± 01.35
IV	142.70 ± 02.12	05.12 ± 00.13	102.30 ± 01.87	12.00 ± 01.65

Result expressed as mean ± SEM. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*

Table 11. Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on the plasma urea and creatinine of treated rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)
I	0.40 ± 00.26	51.28 ± 3.15
II	0.58 ± 00.24	58.03 ± 0.83
III	0.70 ± 00.10^{a+}	65.85 ± 2.65^{b+}
IV	0.88 ± 00.37^{b+}	74.35 ± 02.37^{c+}

Results are expressed as mean ± SEM. $a+$, $b+$ and $c+$ represent significant increases at p<0.05, p<0.01 and p<0.001, respectively, when compared to the control (Group I) values

Table 12. Effect of 14-days of oral toxicity reversibility of 82-2050 mg/kg/day of *NPM* on the plasma urea and creatinine of treated rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)
I	01.14 ± 00.03	33.13 ± 02.37
II	01.17 ± 00.04	36.49 ± 02.37
III	01.11 ± 00.03	39.24 ± 01.84
IV	01.24 ± 00.08	37.09 ± 02.92

Results are expressed as mean ± SEM. I = 10 ml/kg of distilled water; II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*

Effect of subchronic oral treatment with 82-2050 mg/kg/day of NPM on the plasma electrolytes (Na_{+}, K_{+}, Cl_{-} and HCO_{3-}) of treated rats : Single daily oral treatment of rats with 82-2050 mg/kg of *NPM* for 90 days caused significant dose related decreases (p<0.05, p<0.01 and p<0.001) in the plasma levels of Na_{+} and Cl_{-} and a significant dose related increases in the plasma K_{+} while it had no significant alterations in the plasma bicarbonate levels when compared to the control (Group I) values (Table 9).

Effect of 14-days oral toxicity reversibility of NPM on the plasma electrolytes (Na_{+}, K_{+}, Cl_{-} and HCO_{3}) of treated rats: Upon withdrawal of oral treatment with *NPM* for 14 days, there was a non-significant (p>0.05) alterations in the plasma levels of Na_{+}, K_{+}, Cl_{-} and HCO_{3-} when compared to control (Group I) values to levels comparable to the control (Group I) values (Table 10).

Effect of subchronic oral treatment with 82-2050 mg/kg/day of NPM on the plasma urea and creatinine of treated rats: Single, daily oral treatment of rats with 82-2050 mg/kg of *NPM* for 90 days caused significant dose related increases (p<0.05, p<0.01 and p<0.001) in the plasma levels of urea and creatinine when compared to the control (Group I) values) (Table 11).

Effect of 14-days oral toxicity reversibility test of NPM on the plasma urea and creatinine of treated rats: Withdrawal of oral treatment with *NPM* for 14 days was associated with non-significant alterations in the plasma levels of urea and creatinine when compared to the control (Group I) values (Table 12)

Effect of subchronic oral treatment with 82-2050 mg/kg/day of NPM on full blood count parameters of treated rats: Single, daily oral treatment of rats with 40-1000 mg/kg of *NPM* for 90 days caused a significant (p<0.05) dose related increases in RBC, HGB, HCT, MCH, MCHC, PLT and WBC when compared to the control values (Table 6) while causing non-significant (p>0.05) alterations in the white blood cell differentials (% LYM, % EOS, % MON, %BAS and %NEU) when compared to the control values (Table 13).

Effect of 14-days oral toxicity reversibility of NPM on full blood count parameters of treated rats: Withdrawal of oral treatment with *NPM* for 14 days was associated with significant ($p<0.0001$) dose related reductions in %eosinophil and %basophil differentials and significant increases in %lymphocyte differentials when compared to the control (Group I) values (Table 14)

Histopathological studies of the effect of subchronic oral treatment with 82-2050 mg/kg/day and oral toxicity reversibility of NPM on vital body organs of treated rats: The effect of the subchronic oral treatment with 82 mg/kg/day, 410 mg/kg/day and 2050 mg/kg/day of *NPM* on some selected vital body organs in the treated rats are depicted in Figures 1-6 depicting different histological lesions induced by different doses of *NPM* with which the different groups of rats were treated.

Table 13.
Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on the full blood count parameters of treated rats

Parameters	I	II	III	IV
RBC	07.15±00.15	07.67±00.31	07.80±00.27	07.83±0.05^{a+}
HGB	13.36±00.29	14.26±00.26	14.40±00.41	14.80±0.34^{a+}
HCT	44.08±00.55	46.02±00.54^{a+}	46.94±00.33^{b+}	48.12±0.52^{c+}
MCV	71.42±02.13	74.66±01.23	75.00±01.56	76.60±0.45
MCH	26.98±00.51	20.25±00.43	20.66±00.19	21.16±0.18^{a+}
MCHC	26.98±00.57	30.12±00.35^{b+}	31.32±00.45^{c+}	32.38±0.41^{c+}
PLT	640.80±25.62	651.80±48.60	800.00±32.62	855.80±65.60^{a+}
WBC	06.54±00.32	06.98±01.04	08.37±00.47	09.54±0.18^{a+}
%LYM	45.00±00.97	55.10±07.63	54.72±02.36	54.22±05.85
%EOS	03.92±02.19	01.37±00.86	02.70±01.07	02.12±0.54
%BAS	00.42±00.15	01.47±00.94	01.15±00.58	00.27±0.08
%NEUT	40.94±03.21	33.02±05.23	38.58±01.70	46.22±02.53

$^{a+}$, $^{b+}$ and $^{c+}$ represent significant increases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to control (Group I) values. I = control (10 ml/kg of distilled water); II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*; WBC: White Blood Cell; RBC: Red Blood Cell; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Cell Volume; MCH: Mean Cell Hemoglobin; MCHC: Mean Cell Hemoglobin Concentration; PLT: Platelets; LYM: Lymphocytes; NEUT: Neutrophil; BAS: Basophil; EOS: Eosinophil

Table 14.
Effect of subchronic oral treatment with 82-2050 mg/kg/day of *NPM* on the full blood count parameters of treated rats.

Parameters	I	II	III	IV
RBC	07.15±00.15	07.67±00.31	07.80±00.27	07.83±0.05^{a+}
HGB	14.05±00.52	13.73±00.12	14.38±00.26	14.21±00.21
HCT	48.47±00.99	45.78±01.12	48.88±01.03	47.45±00.67
MCV	66.52±00.22	64.03±00.74	65.70±01.21	65.00±01.03
MCH	19.28±00.47	19.04±00.26	19.25±00.26	19.38±00.41
MCHC	28.95±00.65	30.32±00.91	28.93±00.24	29.52±00.67
PLT	808.40±48.06	639.20±17.19	564.50±56.18	571.80±38.12
WBC	06.71±00.47	05.52±00.06	06.08±00.79	06.13±00.63
%LYM	39.82±03.00	69.73±00.78^{c+}	44.03±02.94	73.50±01.14^{c+}
%EOS	04.67±00.23	00.77±00.80^{f-}	00.92±00.10^{f-}	00.80±0.09^{f-}
%BAS	00.52±00.04	00.12±00.05^{f-}	00.22±00.05^{f-}	00.10±0.03^{f-}
%NEUT	47.79±03.41	24.51±00.50^{f-}	47.30±03.91	19.10±00.56^{f-}

$^{a+}$, $^{b+}$ and $^{c+}$ represent significant increases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to control (Group I) values. I = control (10 ml/kg of distilled water); II = 82 mg/kg/day of *NPM*; III= 410 mg/kg/day of *NPM*; IV= 2050 mg/kg/day of *NPM*; WBC: White Blood Cell; RBC: Red Blood Cell; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Cell Volume; MCH: Mean Cell Hemoglobin; MCHC: Mean Cell Hemoglobin Concentration; PLT: Platelets; LYM: Lymphocytes; NEUT: Neutrophil; BAS: Basophil; EOS: Eosinophil

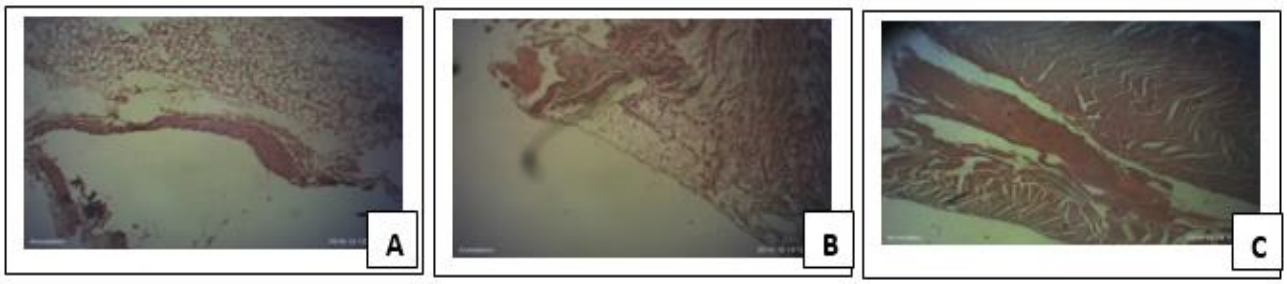

Figure 1.
Photomicrograph of heart from rats treated with: (A) distilled water, (B) 2050 mg/kg/day of *NPM* showing mild vascular congestion and reduced pericardial fatty tissue, and (C) 2050 mg/kg/day of *NPM* showing reduced pericardial fatty tissue and moderate vascular congestion 14 days post-withdrawal of *NPM*. H & E, X100.

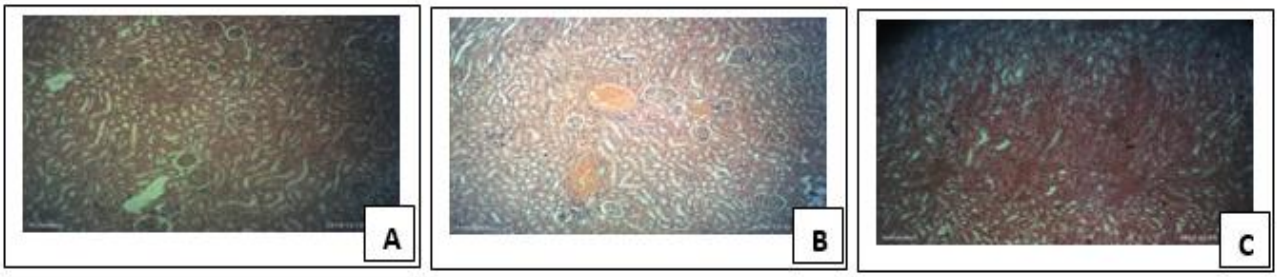

Figure 2.
Photomicrograph of Kidney from rats treated with: (A) 10 ml/kg/day of distilled water for 90 days showing normal glomeruli and renal tubules, (B) 2050 mg/kg/day of *NPM* for 90 days showing remarkable renal vascular congestion and interstitial hemorrhage, and (C) 2050 mg/kg/day of *NPM* for 90 days showing marked renal vascular congestion and interstitial hemorrhage 14-days post-withdrawal of *NPM*. H & E, X100.

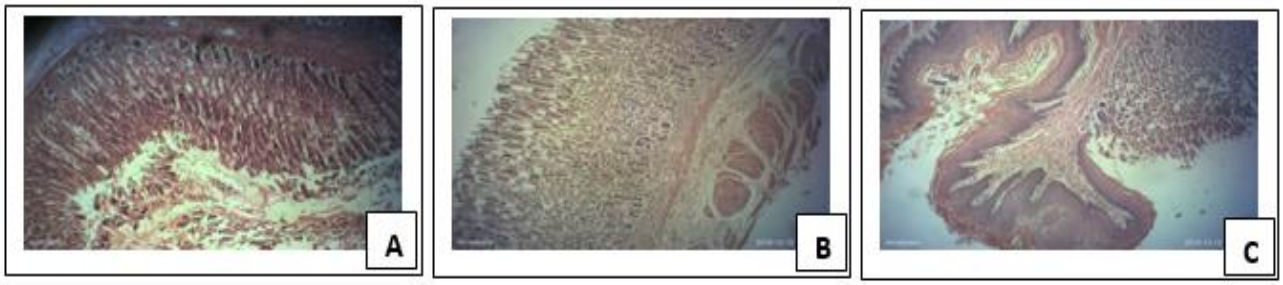

Figure 3.
Photomicrograph of stomach from rats treated with: (A) 10 ml/kg/day of distilled water for 90 days showing normal gastric architecture, (B) 2050 mg/kg/day of *NPM* for 90 days showing some degree of mucosal erosion with infiltration of lamina propria with mild inflammatory cells mostly neutrophils and eosinophils and, (C) 2050 mg/kg/day of *NPM* for 90 days showing some degree of mucosal erosion with infiltration of lamina propria with inflammatory cells mostly neutrophils and eosinophils 14-days post-withdrawal of the herbal mixture. H & E, X400.

Figure 4. Photomicrograph of splenic tissue from rats treated with: (A) 10 ml/kg/day of distilled water for 90 days showing normal splenic architecture, (B) 2050 mg/kg/day of *NPM* for 90 days showing distortion of the lymphoid architecture and distension of the splenic sinuses. and, (C) 2050 mg/kg/day of *NPM* for 90 days still showing distortion of the lymphoid architecture and distension of the splenic sinuses 14-days post-withdrawal of *NPM*. H & E, X400.

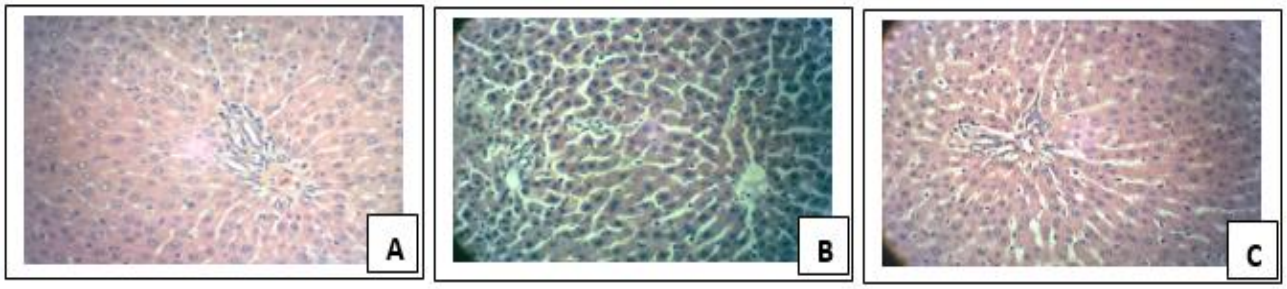

Figure 5. Photomicrograph of liver from rats treated with: (A) 10 ml/kg/day of distilled water for 90 days showing normal hepatic architecture, (B) 2050 mg/kg/day of *NPM* for 90 days showing moderately congested hepatic vessels and sinusoids but normal portal triads and, (C) 2050 mg/kg/day of *NPM* for 90 days showing slightly congested hepatic vessels and sinusoids but normal portal triad and hepatocytes indicating some degree of recovery. H & E, X400.

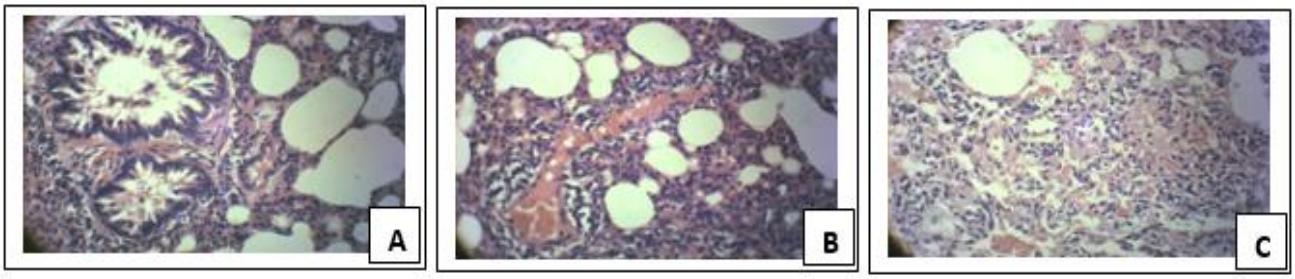

Figure 6. Photomicrograph of lung section from rats treated with: (A) 10 ml/kg/day of distilled water for 90 days showing normal lung tissue architecture, (B) 2050 mg/kg/day of *NPM* for 90 days showing marked congested pulmonary blood vessels and normal alveoli and, (C) 2050 mg/kg/day of *NPM* for 90 days showing persistent congested pulmonary blood vessels but normal alveoli 14-days post-withdrawal of the *NPM*. H & E, X400.

DISCUSSION

Polyherbal combinations are generally believed to provide holistic treatment of human diseases even when the safety profile of such a herbal mixture remains unknown (Sharma *et al.*, 2020). In this present study, the acute and subchronic oral toxicity studies of *NPM* in young nulliparous Wistar rats using anthropometric, biochemical, hematological and histopathological parameters as measured endpoints were conducted using standard scientific procedures.

Single oral treatment of rats with 5000 mg/kg body weight of *NPM* produced no death within the short- and long-term outcomes of the limit dose test of Up and Down Procedure. However, the behavioral toxicity observed include restlessness/agitation, abnormal body posture, generalized body tremor, feed and water refusal within 24 hours post-treatment which gradually subsided after 24 hours post-treatment with full recovery attained by the treated rats 48 hours post-treatment. Thus, *NPM* can be considered orally safe on acute or short-term oral exposure.

Subchronic oral treatment with 82, 410 and 2050 mg/kg/day of *NPM* over the period of 90 days showed that *NPM* did not cause profound alterations in the serum levels of liver enzymes and other liver function parameters indicating safety profile of *NPM* in the treated rats even on prolonged oral exposure. Liver is known to be the main organ of detoxification for most drugs and alterations in its enzyme markers are considered strong indicators of toxicity profile of a drug (Woodman, 1996; David and Hamilton, 2010). Thus, the fact that *NPM* caused no significant alterations in these hepatic enzyme markers suggests that *NPM* is not injurious to the liver since liver injury (hepatotoxicity) is marked by profound elevations in the serum levels of ALT, AST, ALP and at times reduced serum total protein and albumin levels (Giannini *et al.*, 2005; Arika *et al.*, 2016). However, on the renal function parameters measured which included plasma urea and creatinine levels, oral treatments with 82-2050 mg/kg/day of *NPM* for 90 days induced profound elevations in these measured parameters. These findings are suggestive of the potential nephrotoxic effect of prolonged oral exposure to *NPM*, although results of the histopathology of the kidneys of *NPM*-treated rats were corroborative of these biochemical findings showing dose-dependent renal vascular congestions. Alterations in the plasma levels, particularly, profound elevations in these renal function parameters are induced by drugs with nephrotoxic potentials. The mere fact that *NPM* profoundly elevated the plasma levels of the measured renal function parameter coupled with the histopathogical report of associated vascular congestions are strong indications that *NPM* may have a deleterious effect on the renal function upon prolonged exposure to it. However, the nephrotoxic potential of *NPM* could be attributed to the presence of *Cassia sieberiana* which has

previously been reported to have caused significant elevation in the serum creatinine and urea concentration in Wistar rats treated with 20, 60 and 180 mg/kg of the aqueous stem bark extract of *Cassia sieberiana* for 6 weeks (Obidah *et al.,* 2009). Similarly, histopathological reports showed that *NPM* may also have deleterious effects on the heart tissues causing dose-dependent vascular congestions on the heart tissue; on the lung tissues causing interstitial distortion as well as on the stomach causing dose-dependent gastritis in the treated rats. These were also reflected on the relative organ weights especially on the lungs and the stomach where prolonged oral treatment with 82-2050 mg/kg/day of *NPM* induced profound non-dose dependent reductions in the relative organ weights of lungs and stomach of treated rats.

On the hematological parameters, *NPM* significantly improved the full blood counts except for the differential white cell counts which were not significantly altered by prolonged oral treatment of rats with 82-2050 mg/kg/day for 90 days. However, these improvements could be attributed to the presence of *Sorghum bicolor* which has been widely reported to have pronounced hematopoietic effect due to the abundant polyphenols (particularly flavonoids contents (Ogwumike, 2002; Akande *et al.*, 2010b; Benson *et al.*, 2013) although the presence of other constituent plants may also have contributed to the improved hematological profile as recorded in this study. Another worthy observation is the tendency towards hypercoagulability with 2080 mg/kg/day of the herbal mixture which was strongly indicated by thrombocytosis (significant elevation in the platelet counts in the blood) which is closely regulated by the kidney- and liver-producing thrombopoeitin (Hitchcock and Kaushansky, 2014). Literature has shown a strong and direct correlation between thrombocytosis and vascular thromboembolism and stroke resulting from increased platelet aggregation (Rinder *et al.*, 1998; Khorshed *et al.*, 2007; Chu *et al.*, 2010; Koupenova *et al.*, 2017).

Another significant finding of this study is that *NPM* caused non-significant alterations (be it loss or gain) the average body weights and relative organ weight of the treated rats (except that of stomach and lungs which were significantly reduced although in non-dose dependently). These findings may be related to the absence of tannins and phlobatannin in the *NPM* which have been reported to induce weight loss in extract/polyherbal formula abundantly rich in this secondary metabolite due to their appetite inhibiting effect and anemic effect (Chung *et al.*, 1998; Amesa *et al.*, 2018; Valenti *et al.*, 2019). However, in the oral toxicity reversibility studies, all of the measured biochemical (liver and renal function parameters) and hematological changes induced by chronic oral treatment with 82-2050 mg/kg/day of *NPM* were reversed upon stoppage of the oral treatment (reversibility test) of the herbal mixture for 14 days but the "tell-tale" signs of histological lesions in the heart, kidneys, stomach and spleen were still remarkable despite withdrawal of the herbal mixture.

In conclusion, *NPM* although widely consumed to traditionally relieve pains associated with gastrointestinal disorders such as rectal prolapse and hemorrhoids, menstrual and waist pain, it may not be considered safe for consumption on long term use as it showed tendency to be nephrotoxic and cause gastritis and deleterious effects on other body organs likes the lungs and spleen on prolonged oral exposure, although, our studies showed that its prolonged oral consumption caused improved hematological profile in the treated rats. While *NPM* may modulate biochemical and hematological balance in the system, patients with occult or underlying renal diseases or reduced renal function should exercise caution in its use as it may result into full blown renal failure.

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Antidepressant-Like Effect of Ethanol Extract of *Blighia Unijugata* Bak. (Sapindaceae) Leaves in Acute and Chronic Models of Depression in Mice

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Summary: *Blighia unijugata* (Sapindaceae) is an indigenous tree belonging to the tropical forests of West Africa. It is called "Ako Isin" by the Yoruba people of Southern-Western part of Nigeria, where it is among plants used traditionally in the management of depressive psychosis. The aim of this present study was to evaluate the anti-depressant activity of ethanol extract of *Blighia unijugata* leaves *in-vivo* using acute and chronic experimental models of depression. The antidepressant activity of ethanol extract of *B. unijugata* leaves was investigated using acute and chronic unpredictable mild stress. Depression tests used included forced swimming, tail suspension, yohimbine induced lethality and reserpine induced depression tests. Oxidative stress markers were also assessed in the brain homogenates after chronic unpredictable mild stress. The LD_{50} via oral route of administration was 1414 mg/kg. The results showed that, *B. unijugata* produced significant reduction in immobility time in forced swimming and tail suspension tests without stimulating in locomotor activity in open field test. It was also found that *B. unijugata* significantly reversed diarrhea, ptosis and hypothermia in reserpine model of depression. 2.5 mg/kg *B. unijugata* potentiated yohimbine induced lethality in mice and also reduced the oxidative stress markers. The ethanol extract of *B. unijugata* leaves possessed antidepressant action, thus justifying its use in the management of mental illness.

Keywords*:* Flavonoids, Blighia unijugata*; antidepressant; immobility; yohimbine; reserpine; ptosis.*

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Introduction

Depression is a common psychiatric disorder, affecting approximately 20% of the worldwide population (Gao *et al.,* 2013). The World Health Organization (2009) predicts that it will become the second leading cause of premature death or disability worldwide by the year 2020 (Wager-Smith and Markou, 2011). Chronic psychosocial stress majorly predisposes most susceptible individuals to depression (De Kloet et al., 2005; Siegrist, 2008). Sadness, loss of interest or pleasure, feelings of low self-worth, disturbed sleep and poor concentration are some of the characteristics of depression (Chowdhury and Juvekar, 2014). The primary mechanism of this disorder include depletion of monoamines, oxidative stress and hyperactivity of HPA-axis (Dhingra and Bhankher, 2014). Although, the pathophysiology of this mental illness is not very clear, increasing evidence support the hypothesis that neurotransmitters deficiencies are extraordinarily important to its etiology; this has made the prescription of monoamines oxidase inhibitors and selective serotonin reuptake inhibitors (SSRIs) more frequent for patients suffering depression (Shen et al., 2016). Most of the drugs in use currently, target this deficit, which is empirical, symptom oriented and not disease specific. Also, these drugs have numerous limitations including unpleasant side effects (Nash and Nut, 2005). This gave rise to the use of plants as a therapy for this disorder; especially those with little or no side effects.

Reports by World Health Organization (1985), reveal that about 80% of the people living in developing countries almost exclusively use herbal medicine for their primary health care needs. The screening of these herbal medicines which are mostly of plant origin is a potential source of novel drug prototypes (Rabe and van Staden, 1997; Afolayan, 2003). Plants are the most natural and accessible sources of therapeutically active biological compounds. *Blighia Unijgata* belongs to Sapindaceae plant family; it is widely distributed with 136 genera and 2000 species (Urdampilleta et al., 2005). Many species in this family have been reported to possess a number of biological and pharmacological

activities (Basile et al., 2005). *Blighia unijugata*, (BU) has been reportedly used traditionally in the management of psychosis in the Southern-Western part of Nigeria (Sofidiya et al., 2011).

Significantly, there is paucity of studies investigating BU leaf extract's antidepressant activity and its probable extended pharmacological effect (side effects) which is a bane of drugs currently used for the treatment of depression. We therefore undertook this study considering the profile of side effects of currently used antidepressants and the paucity of antidepressant studies on BU. The aim of the study was to investigate the antidepressant-like effect of the ethanol extract of *Blighia Unijgata* leaves in acute and chronic models of depression.

Materials and Methods

Plant materials

Fresh leaves of *Blighia Unijugata* were collected at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves was also authenticated at the FRIN, where a voucher specimen with the number (FHI 110119) was deposited.

Preparation of plant material and drugs

The leaves were air-dried, pulverized and 100g was macerated for 48 hours in 1.75 L of 50% ethanol. The ethanol extract was decanted, filtered and concentrated under a rotary evaporator at the pharmaceutical chemistry laboratory of the University of Ibadan. The concentrated extract of *Blighia Unijugata* (BU) was dried and stored in a desiccator. On each day of the experiment, the extract obtained was freshly dissolved in distilled water which served as vehicle.

Experimental Animals

Male Swiss mice (20-25 g) were obtained from the Animal Centre, College of Medicine, University of Ibadan, Nigeria, and were housed in plastic cages at room temperature. They were fed with balanced rodent pellet diet and water *ad libitum*. The animals were acclimatized for at least 1 week before being used for the experiments. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Drug and Chemicals

Imipramine (Sigma-Aldrich, St. Louis, USA), Thiobuturic acid (TBA), Trichoroacetic acid (TCA), DTNB reagent, phosphate buffer, ethanol and distilled water.

Acute toxicity test

The method described by Lorke (1983) was used to determine the LD_{50} which is the index of acute toxicity. Male Swiss mice (20 - 25 g) were used for the test. Briefly, this method involved an initial dose finding procedure, in which the mice were divided into three groups of three mice each. Doses of BU (10, 100 and 1000 mg/Kg) were administered intraperitoneally, one dose for each group. The treated animals were monitored for mortality and general behavior for 24 hours. From the result of the dose finding step, four higher doses of BU (2000, 3000, 4000, 5000 mg/Kg) were chosen and administered (i.p.) respectively to four groups of one mouse per group. The treated animals were monitored for 24 hours. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

Tail Suspension Test (TST)

Tail Suspension Test is a commonly employed behavioral model for screening antidepressant activity in mice (Steru *et al*., 1985). The total duration of immobility following tail suspension was measured using the model for evaluating potential antidepressants postulated by Rodrigues *et al.*, (2002). Briefly, each mouse was individually suspended 50 cm above the floor by the tip of the tail (approximately 1 cm) adhered to a lever. The mouse under testing was quarantined during the test duration. A total testing period of 6 minutes was allowed. After the first 2 minutes following suspension of the mouse by the tail; the duration of immobility was manually recorded using a stopwatch during the next 4 minutes of test. The mouse was considered to be immobile when it did not show any body movement, hung passively and was completely motionless. The test was conducted between 8 am -12 pm in a quiet room to avoid change in biological rhythm and disturbance. The protocol was repeated for each mouse in the experimental groups consisting of 5 mice. The groups are as follows: group 1 received distilled water (0.2 mL/20 g), groups 2-4 received BU (1.25, 2.5 and 5 mg/Kg) and group 5 received imipramine (10 mg/Kg). All treatments were administered 30 minutes before the test.

Forced Swimming test (FST)

Forced Swim Test is a behavioral test for assessment of antidepressant activity of compounds. The test was performed according to the procedure described by Porsolt *et al*., (1997). The rodents were placed individually in Plexiglas cylinders (40 cm in height, 18 cm in diameter) filled with water (25 ◦C) up to 15 cm. Two minutes pre-swimming period was followed later by 4 minutes test period during which the total immobility time was measured. The mice were considered immobile when they made no further attempts to escape for necessary movements to keep their heads above the water. The absence of hind leg movement was recorded as immobility by stopwatch during the exposures. The water in the cylinder was changed before every trial and the mice were towel dried before being returned into their home cage after the swimming sessions.

Locomotor activity in the Open Field

Motor activity was measured in the open field apparatus (white Plexiglass box measuring 28 cm × 28cm × 25cm, with the floor equally divided into 16 equal squares marked with painted black grid). Thirty minutes after the administration of an extract or standard drug, each mouse was placed separately in the centre of the box, and the number of squares crossed by all four paws were counted for 5 minutes. The floor of the open field apparatus was cleaned with 70% ethanol and allowed a 5 minutes interval before the next animal was assessed (Akanmu *et al.,* 2011).

Yohimbine Induced Lethality Test

The involvement of noradrenergic system in the antidepressant-like effect of the extract was evaluated using yohimbine-induced lethality test. The test was performed as described by Vogel & Vogel (1997). Fifty (50) mice were assigned into five groups (n=10). Group 1 received distilled water (10 mL/Kg); groups 2-4 received different doses (1.25, 2.5 and 5.0 mg/Kg; i.p.) of leaf extract of BU; group 5 received imipramine (10 mg/Kg; i.p.). All the treatments were done thirty minutes prior to administration of Yohimbine (35 mg/kg; i.p.). The number of death and percentage lethality was calculated 24 hours after the injection of Yohimbine.

Reserpine-induced hypothermia, ptosis and diarrhea in mice

The test of reserpine-induced hypothermia, ptosis and diarrhea were in accordance with those of Bourin *et al.,* (1983). The mice were administered reserpine (2.5 mg/kg) 30 minutes after treatment with either distilled water, BU or imipiramine. The treatment was performed in five groups of male mice (n=5). Group 1 was given distilled water (0.2 mL/20 g), while groups (2-4) were given different doses of BU (1.25, 2.5, 5 mg/Kg); and group 5 was give imipramine (10 mg/Kg). The rectal temperature were recorded at 0, 1, 2, 3 and 4 hours, respectively, after the administration of reserpine. The degree ptosis was evaluated after 4 hours according to the following rating scale: eyes open = 0, quarter closed =1, eyes half closed = 2, eyes three-quarters closed = 3 and eyes completely closed = 4. Body temperature was measured using a rectal thermometer. The probe of the thermometer was inserted 1.5cm into the rectum. The pre-drug recording served as the reference point for the determination of temperature changes (Parimaladevi *et al.,* 2003). Diarrhea was evaluated as number of droppings at time points.

Chronic Unpredictable Mild Stress

Animals were subjected to various stress paradigms once a day, thirty minutes after treatment for a period of 2 weeks as described by (Kumar et al., 2011). Mice were randomly distributed to 6 groups (n=5). Groups 1-2 were administered 10 mL/Kg distilled water (vehicle), groups 3 – 5 were administered BU (1.25, 2.5, and 5.0 mg/Kg) and group 6 was administered imipramine (10 mg/Kg). The mice in group 1 were not stressed, while those in groups 2 – 6 were exposed to various stress conditions over a period of two weeks. Typical stressors included overnight illumination, periods of food or water restriction, cage tilt, and isolation or crowded housing. All administration was done by intraperitoneal route. Behavioral testing was done in independent groups of mice on the 15th day. Tail Suspension Test (TST) and Open Field Test (OFT) were the models employed for evaluation of the presence of antidepressant activity.

Biochemical assay

Determination of brain glutathione (GSH) concentration

The animals were sacrificed under ether anesthesia and the brains were rapidly removed. Thereafter, half of the whole brain were weighed and homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4). Each brain tissue homogenates was separated into two portions for the different biochemical assays. Aliquots of brain homogenates of individual mouse in the respective treatment groups were taken and GSH concentration was determined using the method of Moron *et al.,* (1979). Equal volume (0.4 ml) of brain supernatant and 20% trichloroacetic acid (TCA) (0.4 ml) were mixed and then centrifuged using centrifuge at 2,000 rpm for 10 min. The supernatant (0.25 ml) was added to 2 ml of 0.6 mM 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) and the final volume was made up to 3 ml with phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm against blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues are expressed as micromoles per gram tissue (µmol/g tissue).

Estimation of lipid peroxidation

Lipid peroxidation (LPO) was determined by estimating malodialdeyde (MDA) levels as described by Ohkawa et al., (1979). MDA and other aldehydes have been identified as products of lipids that react with TBA to give a pink coloured species that absorbs visible light spectrum at 532 nm. The method involved heating of biological samples with TBA reagent for 20 mins in a boiling water bath. TBA reagent contain 20% TCA, 0.5% TBA and 2.5 N HCl. After cooling, the solution was centrifuged at 2,000 rpm for 10 mins and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents minus the biological sample. The MDA equivalents of the sample were calculated using an extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1}.

Statistical Analysis

Data were analysed using Graph Pad Prism version 5.0 and expressed as mean ± S.E.M. Statistical analysis

was done using one-way ANOVA followed by Newman-keuls post- hoc test. P values < 0.05 were considered statistically significant.

RESULT

Acute toxicity test: No lethality/mortality was recorded when doses as high as 1000 mg/kg was given to the mice. The mouse administered the dose 2000 mg/Kg died, and this was used along with the 1000 mg/Kg to calculate the LD_{50}. The LD_{50} of crude extract of *Blighia Unijugata* in mice was found to be 1414 mg/Kg; i. p. body weight.

BU reduced the locomotor activity of mice in OFT: BU at 5.0 mg/kg significantly reduced [F (4, 29) = 4.942, p< 0.05] line crossing activity in the OFT compared to vehicle (Figure 1).

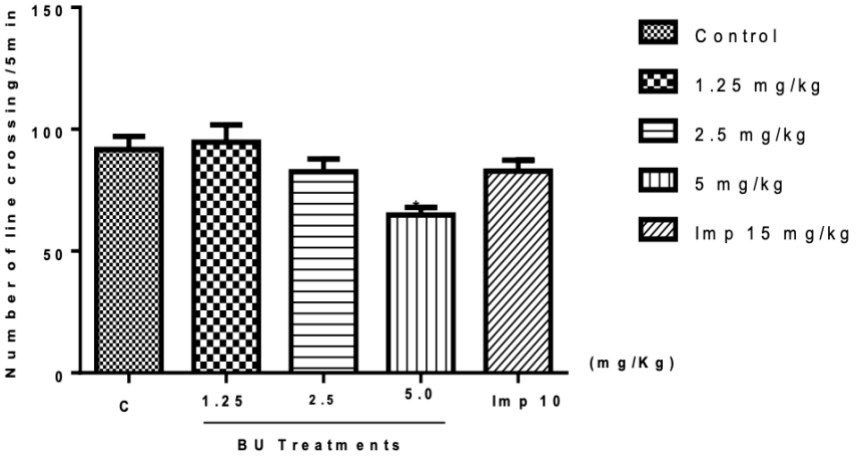

Figure 1:
Effect of BU the locomotor activity of mice in OFT *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

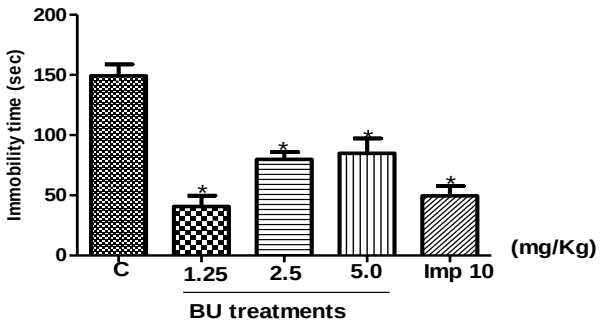

Figure 2:
Effect of BU on immobility time of mice in FST *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

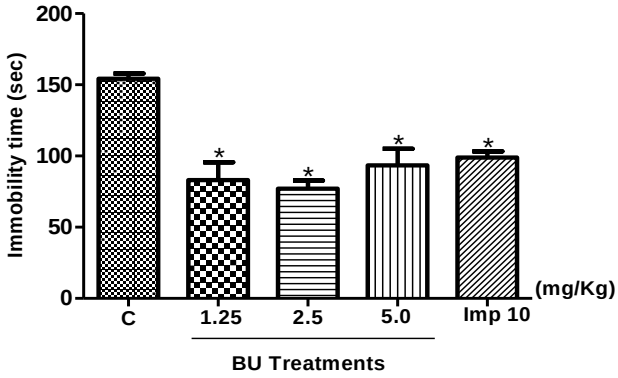

Figure 3:
BU reduced the immobility time of mice in TST *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

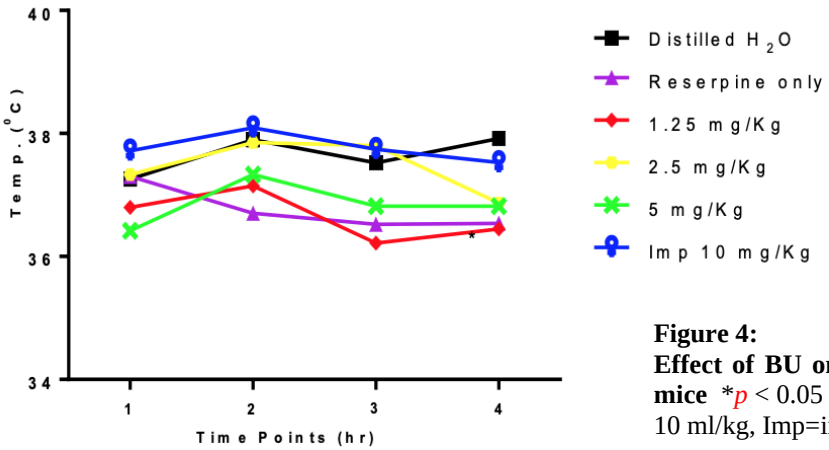

Figure 4:
Effect of BU on reserpine induced hypothermia in mice *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

BU reduced the immobility time of mice in FST: The administration of BU (1.25, 2.5 and 5.0 mg/Kg, p.o.) and imipiramine (10 mg/Kg) significantly reduced [F (4, 29) = 21.25, p< 0.05) the immobility time in the forced swim test (Figure 2)

BU reduced the immobility time of mice in TST: The administration of BU (1.25, 2.5 and 5.0. mg/kg, p.o.) and imipiramine (10 mg/Kg; i.p.) significantly reduced [F (4, 29) = 13.28, p< 0.05) the immobility time in the tail suspension test (Figure 3).

BU reverses reserpine induced hypothermia in mice

The administration of BU (2.5 and 5.0 mg/Kg) prevent reserpine induced hypothermia in mice when compared to reserpine only. BU 2.5 mg/Kg and imipiramine 10mg/Kg and significantly [F (5, 29) = 3.992, p< 0.05) reversed reserpine induced hypothermia (Figure 4)

BU reverses the degree of ptosis in reserpine-induced depression

The administration of BU (1.25, 2.5 and 5.0 mg/Kg) and imipramine (10 mg/Kg) significantly reversed the degree of ptosis during reserpine-induced depression (Table 1).

Table 1:
Effect of ethanol extract of *B. Unijugata* leaves on ptosis and diarrhea in reserpine-induced depression
*P<0.05

Treatment	Score of Ptosis	Diarrhea
Vehicle (10 ml/kg)	1.75±0.23	3.43±0.83
BU (1.25 mg/kg)	0.67±0.17*	1.63±0.40*
BU (2.5 mg/kg)	0.79±0.23*	1.96±0.57*
BU (5.0 mg/kg)	0.167±0.07*	1.67±0.49*
Imipramine (10 mg/kg)	0.67±0.33*	1.33±0.33*

BU reverses score of diarrhea in reserpine-induced depression

The administration of BU (1.25, 2.5 and 5.0 mg/Kg, i.p.) and imipramine (10 mg/Kg) significantly reversed the degree of diarrhea during reserpine-induced depression (Table 1).

Table 2. Effect of ethanol extract of *Blighia Unijugata* leaves on Yohimbine Lethality Test

Treatment	Number of death (n)	% Mortality
Vehicle (10 ml/kg)	2/10	20
BU (1.25 mg/kg)	0/10	0
BU (2.5 mg/kg)	6/10	60*
BU (5.0 mg/kg)	2/10	20
Imipramine (10 mg/kg)	7/10	70*

*P<0.05

Effect of ethanol extract of BU leaves on Yohimbine lethality

2.5 mg/Kg BU and 10 mg/Kg imipiramine produced a significant increase in the number of deaths (P < 0.05) as compared with control (Table 2).

Effect of BU on locomotor activity of mice subjected to chronic unpredictable mild stress

Chronic unpredictable mild stress significantly decreased the locomotor activity in stressed mice as compared to vehicle-treated unstressed control. Imipiramine (10 mg/Kg) significantly (p < 0.001) decreased the immobility period as compared to stressed mice. The administration of BU (1.25- 5 mg/Kg) did not significantly decrease the locomotor activity when compared with stressed control mice (Figure 5).

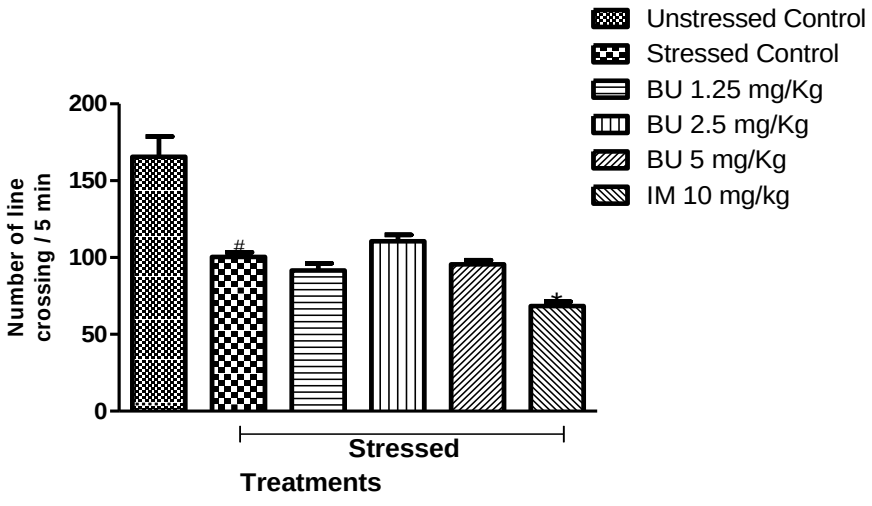

Figure 5:
Effect of BU on locomotor activity in mice subjected to CUMS * indicates significant difference from the stressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$ Control 10 ml/kg, Imp=imipiramine

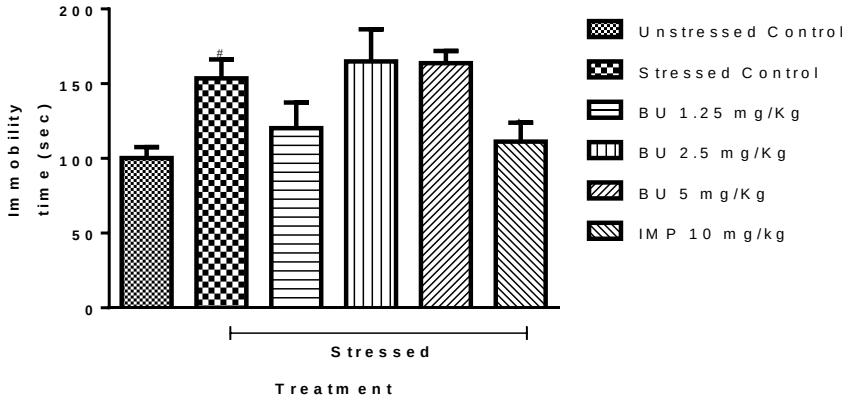

Figure 6:
Effect of BU on immobility time in mice subjected to CUMS * indicates significant difference from the stressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$

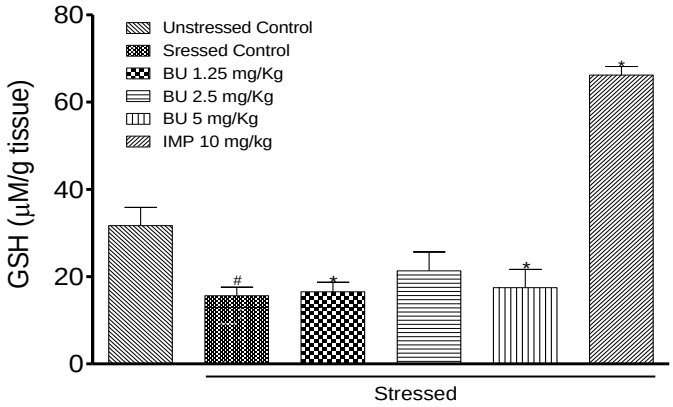

Figure 7:
Effect of BU on brain Glutathione Levels in CUMS * indicates significant difference from the unstressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$

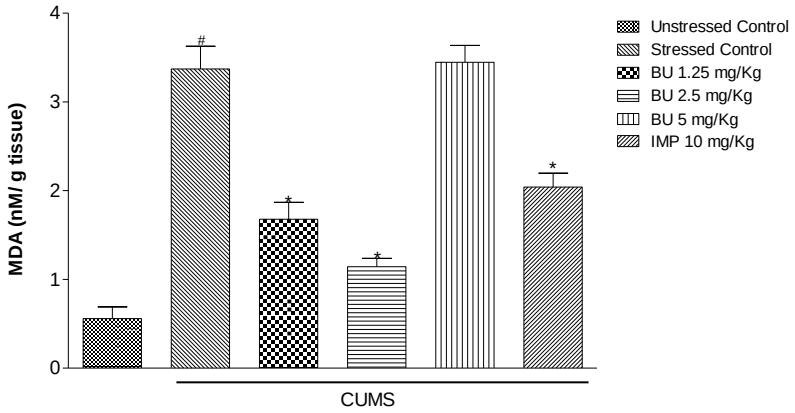

Figure 8:
Effect of BU on brain Malondialdehyde Levels * indicates significant difference from the unstressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$

Effect of BU on immobility time in TST of mice subjected to CUMS

Chronic unpredictable mild stress significantly increased the immobility time in stressed mice as compared to vehicle-treated unstressed control. The administration of BU (1.25- 5 mg/Kg) and imipiramine (10 mg/Kg) did not significantly decrease the immobility time as compared to stressed control mice (Figure 6).

Effect of BU on brain Glutathione Levels

GSH levels were significantly ($p < 0.001$) decreased in brains of vehicle-treated stressed mice when compared with unstressed control (Figure 7). BU (1.25, 2.5 and 5mg/Kg) and imipramine (10 mg/kg) produced a significant ($p < 0.001$) increase in GSH levels in respective treated mice in comparison with stressed control mice.

Effect of BU on brain Malondialdehyde Levels

Malondialdehyde levels were significantly ($p < 0.001$) increased in brains of vehicle-treated stressed mice compared with unstressed control (Figure 8). BU (1.25, 2.5 and 5mg/Kg) and imipramine (10 mg/Kg) produced a significant ($p < 0.001$) decrease in MDA levels in respective treated mice in comparison with stressed control mice.

DISCUSSION

Behavioural studies have been shown to play an important part in the evaluation and development of antidepressant drugs (Xu, et al., 2008). The behavioral paradigms used in the present study include Forced Swim Test (FST), Tail Suspension Test (TST) and open field test. Yohimbine lethality test and reserpine induced depression were also used to investigate the probable mechanism of action. Behavioural and biochemical changes were also studied after inducing chronic depression using Chronic Unpredictable Mild Stress (CUMS). The CUMS was used to determine the underlying factors of depression which may involve the generation of free radicals such as reactive oxygen species. The study established that the acute lethal dose (LD50) of the ethanol extract of *Blighia Unijugata* (intraperitoneal route) is 1414 mg/kg using the method described by Lorke (1983). It could therefore be assumed to have a wide safety margin especially in use as a potential candidate for antidepressant drug discovery. Jebunnessa et al. (2009) extracted alkaloids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones from the leaves of *Blighia Unijugata*.

The immobility displayed by rodents subjected to unavoidable and inescapable stress (in FST and TST) is postulated as reflecting behavioural despair which may reflect depressive disorder in humans (Porsolt *et al.*, 1977). This study revealed that ethanol extract of *Blighia Unijugata* (BU) produces a statistically significant reduction in immobility time in FST and TST. Imipramine, an antidepressant, expectedly produced significant reduction in the immobility time of the tested rodents. It has been established that immobility is reduced by a variety of therapeutically active antidepressants as tricyclics, monoamine oxidase inhibitors, and other new antidepressants (Cryan and Lucki, 2000). The capacity of BU to also trigger a reduction in immobility time suggests that it may possess antidepressant activity.

The possible attribution of the antidepressant effects of test substances to stimulant effect in behavioural models of depression are usually characterized by locomotor activity test (open field test) (Bourin *et al*., 2001). Amphetamines, convulsants and anticholinergic are some compounds which can enhance locomotor activity or cause hyperkinesias in OFT and still produce false positive results in FST and TST (Butterweck *et al*., 2003). Antidepressants and psycho-stimulants are usually discriminated by increased locomotor activity in OFT (Borsini and Meli, 1988).

The possible mechanism underlying the antidepressant activity of BU, was evaluated using the reserpine induced depression reversal test. Existing studies have revealed that reserpine can cause the depletion of amine stores and irreversibly inhibit the vesicular uptake of monoamines. The resulting reduction level of monoamines in the brain is an underlying factor in pathophysiology of depression; and it leads to physiological effects such as diarrhoea, ptosis and hypothermia which have all been associated with reserpine induced depression (Bourin *et al*., 1983). The major classes of antidepressant drugs have reversed or inhibited these syndromes associated with reserpine induced depression. BU (2.5 and 5.0 mg/kg) and imipramine (10 mg/kg) have been observed to significantly reverse hypothermia induced by reserpine. The degree of ptosis and diarrhea were also significantly reversed by BU and imipramine when compared with the control. BU significantly antagonized the clinical observations induced by reserpine. BU might be mediated via the monoamine pathway to trigger the mechanism of its antidepressant-like effect.

To identify neurotransmitter systems that may be involved in the mechanism of actions of antidepressant drugs, the yohimbine induced lethality test is recommended (Leonard *et al*, 1986). Yohimbine is an α_2 adrenergic antagonist which is responsible for increased sympathetic discharge in the peripheral and in the central nervous system. As an antagonist, it also increases the level of serotonin and other biogenic amines when it inhibits the negative feedback regulation of their release (Blier and Montigny, 1994). All currently approved antidepressants (ADs) increase the synaptic availability of one or more of the biogenic amine transmitters: noradrenaline, dopamine, and serotonin. The proposed mechanism of antidepressant drugs in potentiation of yohimbine induced lethality is via involvement of noradrenaline. BU at 2.5 mg/kg potentiated the effect of yohimbine, which resulted in 60% mortality as compared with the control (20% mortality). This effect might either be via inhibition of reuptake or inactivation of monoamine oxidase.

Chronic Unpredictable Mild Stress (CUMS) induced depression is considered the most valid animal model of depressive behavior observed in humans after a long-term exposure to multiple stressors (Willner, 2005). Cortisol (stress hormone) is released in excess via the activation of the hypothalamic pituitary adrenal axis inducing a damage to the dopaminergic, serotoninergic or glutamatergic neurons (Vyas et al, 2016). The consequence of all these changes is reduction in the size of the hippocampus and the frontal cortex, which is characteristic for patients with

severe depression (De Andrade et al., 2013). Animal studies in CUMS model have shown that long-acting stressors cause atrophy of hippocampal pyramidal cells and impair the neurogenesis resulting in a reduction in size (Drevets et al., 2001). The administration of BU (1.25, 2.5, 5 mg/kg) (14-day) reverses the structural and functional changes in the hippocampus and the frontal cortex induced by different chronic stressors. BU might increase the size of the hippocampus via enhancement of neurogenesis in the hippocampus and frontal cortex. Imipramine was responsible for the reversal of the hippocampal atrophy and the inhibition of neurogenesis in the hippocampus and in the cortex.

Also, CUMS impairs the antioxidant status of brain tissue due to excessive production of reactive oxygen species (Bilici *et al.*, 2001). Reactive oxygen species (ROS) plays a vital role in the pathogenesis of neuropsychiatric disorders that cause oxidative damage to macromolecules (lipids, proteins and DNA) and result in neuronal dysfunction and depression (Esch et al., 2002). Lipid peroxidation and other antioxidant enzymes may be biomarkers of major depression due to the fact that they return to normal levels after treatment with antidepressants (Bilici et al., 2001). In this study, 14 days of successive exposure to unpredictable mild stress using different stressors resulted in the reduction in GSH and increased in the amount of MDA in stress exposed mice. BU (2.5 mg/kg) and imipramine significantly increased GSH level in the brain. BU (1.25 and 2.5 mg/kg) and imipramine also significantly reduced the brain level of MDA. Thus, BU might possess a neuroprotective effect against oxidative stress in CUMS-induced depression. Some species of plants such as *Bacopa monneira, Withania somnifera* and *Asparagus racemosus*, have been reported to have antidepressant-like properties attributable to their antioxidant activity (Sairam et al., 2002; Bhattacharya et al., 2000). Therefore, it is possible that the antioxidant activity of the BU may contribute to its antidepressant-like effect.

Finally, the results of this study showed that the leaves of *Blighia unijugata* possess antidepressant properties. This neuropharmacological property is possibly mediated through the facilitation of noradrenergic pathways and antioxidant activity. However, considering the limitations of the models used, clinical studies involving humans may be need to be carried out to further confirm the results of our study.

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Effect of *Byrsocarpus Coccineus (Connaraceae)* Aqueous Leaf Extract on Pancreatic Islets Volume in Type 2 Diabetic Male Wistar Rats.

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Summary: Diabetes mellitus is a devastating illness associated with alterations in the pancreatic islet of Langerhans and the islet volume constitutes a significant variable in diabetic investigations. This study investigates the volume of pancreatic islets in type 2 diabetic rats following treatment with aqueous extract *Byrsocarpus coccineus* leaves. Twenty-five male Wistar rats weighing between 100-120g were divided into five groups. Group 1 served as the normal control given distilled water while type 2 diabetes mellitus was induced by a high fat diet feeding for five weeks and a single dose of streptozotocin (35mg/kg, *i.p.*) in groups 2,3,4 and 5. After confirmation of diabetes, animals in groups 2,3,4 and 5 were administered normal saline, 50 mg/kg metformin, 800 mg/kg *Byrsocarpus coccineus* leaves leaf extract (BCLE) and 400 mg/kg BCLE respectively for 21 days. The pancreas was harvested, fixed in neutral buffered formalin and processed for stereological and histological analysis. Isotropic uniform random samples were obtained with the orientator grid. Serial sections were cut with a rotatory microtome and stained with Haematoxylin and Eosin. Pancreatic islet volumes were measured with the aid of a Cavalieri estimator grid. The result showed significant ($p<0.05$) increase in the blood glucose level of the diabetic control group, when compared to the normal control. But blood glucose levels from groups 4 and 5 were significantly ($p<0.05$) decreased when compared to the diabetic control. Pancreatic islet volume estimations showed a significant decrease in the diabetic control group when compared to the normal control ($p<0.05$), while pancreatic islet volumes in groups 3, 4 and 5 were significantly increased when compared to the diabetic control group ($p<0.05$). In a likewise manner the histology of the pancreas of the diabetic control shows damaged pancreatic islet cells and surrounding tissue that was reduced in all the treated group. In conclusion the aqueous extract of *Byrsocarpus coccineus* has shown an anti- hyperglycaemic effect in the experimental rats and increased the volumes of the pancreatic islet cells as well as ameliorated the pathological changes in the pancreas.

Keywords*: Byrsocarpus coccineus*, Pancreatic islet volume, Stereology, Type 2 diabetes mellitus

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INTRODUCTION

Diabetes mellitus is the most common endocrine disorder in man, is estimated to affect 4% of the world's population; a doubling of this figure is expected in the near future, especially in the African and Asian continents (Engelgau *et al*., 2003). Most Nigerians living with diabetes have suboptimal glycemic control and have chronic complications (Chinenye and Young 2011). In Nigeria, the national prevalence of diabetes mellitus was estimated to be 6.8% in adults older than 40 years (Abubakaria and Bhopalb, 2008). Earlier in a survey of type 2 diabetes mellitus in Giwa and Markarfi local government areas, both sub-urban communities in Kaduna state in the northern part of Nigeria showed a prevalence rate of 1.6% (Bakari *et al*., 1999) and Dahiru *et.al.* (2008), reported prevalence rate of 2.0% in Dakace village, a semi-urban community in Zaria local government area in Kaduna state. A study of the prevalence of diabetes mellitus in Nigeria showed that type 2 diabetes mellitus is the most common type of diabetes accounting for about 90% of cases (Familoni *et al*., 2008).

The endocrine islets are scattered among the exocrine acini in the pancreas of human and mammals, hormone-secreting cells containing insulin, glucagon, somatostatin, and pancreatic polypeptide are present in the islets of Langerhans. The insulin containing cells are most numerous (60-80% of the islets) and are generally located in the central part of the islets (Knop *et al.*, 2010). Normal islet cell mass and function was initially, associated with type 1 diabetes, which is

characterised by the loss of β-cell mass due to loss of functional β-cell mass is now also associated with type 2 diabetes (T2D) (Bonner-Weir and O'Brien, 2018). Studies have observed that type 2 diabetes in human and rodent pancreas specimens show a loss of desmosomes and adherens junctions between islet and acinar cells, due to fibrosis and remodeling of the islet-acinar interface, that may result in an impaired function (Hayden *et al.,* 2008)

Byrsocarpus coccineus is a shrub plant that belongs to a family connaracea and commonly found across west and tropical Africa. Scientific study of *Byrsocarpus coccineus* revealed that the aqueous leaf extract possesses anti-inflammatory (Akindele *et al*., 2007), anxiolytic activity (Akindele *et al*., 2010) and anti-oxidant effect (Andrew *et al*., 2017). A number of study have reported the anti-hyperglyceamic effect of *Byrsocarpus coccineus (Dada et al., 2013*) in this regard the changes in pancreatic islets volume of diabetic animals following treatment with aqueous extract *Byrsocarpus coccineus* (leaves) may be an important consideration since, the β-cell composition in human islets has been reported as percentage on the basis of cell number or cell volume (Chen *et al.,* 2017) Additionally, studies by Dada *et al*. (2013) reported, an increased insulin level with the high dose of extract *Byrsocarpus coccineus*. Also enhanced insulin secretion concurs with β-cell function. Therefore, the analysis of the volume of pancreatic islets in diabetic rats treated with *Byrsocarpus coccineus* will further substantiate the anti-diabetic effect of the plant.

MATERIALS AND METHODS

Drugs, chemicals, reagents and other materials

Metformin tablets (Pharmatex Nigeria Ltd), dextrose solution, Streptozotocin (STZ) (Sigma-Aldrich, St Louis, USA). Glucose test kit (Accu-Check performa; Roche diagnostics Germany), Animal-derived fat, (Zaria Abattoir after Veterinary screening). Ketamine hydrochloride injection 50mg/ml; Batch no: N-5629, Manufactured by Kwality Pharmaceuticals PVT. LTD. India. Neutral buffered formalin, Orientator 44 grid, Cavalieri Estimator grid and Rotatory Microtome.

Plant material and extraction

The leaves of *Brysocarpus coccineus* were collected from the Galadimawa / Birnin-Gwari road in Giwa Local Government Area of Kaduna state, Nigeria. The leaves were identified and authenticated in the department of Botany, Ahmadu Bello University, Zaria. A voucher specimen with number 590 was assigned. Plant material was shade-dried, processed and aqueous extraction was done using maceration method as described by Brian *et al*. (1975) in the Department of Pharmacognosy, Ahamdu Bello University, Zaria, Kaduna state, Nigeria. About 150g of *Byrsocarpus coccineus* leaves yielded 15.43g of extract.

Animals and Animal Handling

Twenty-five apparently healthy adult male Wistar rats weighing between 100g- 120g were used. They were housed in plastic cages under good laboratory conditions. The rats were allowed to acclimatize for two (2) weeks before commencement of the research. Animals were fed with pelletized form of Growers' mash (protein 13%, fat 8%, fiber 15%, calcium 0.9%, phosphorus 0.35% and metabolisable energy – 2550Kcal/Kg) purchased from vital feeds, Zaria-Kaduna state, and water *ad libitum*. Rats were handled humanely according to the laws guiding the use of laboratory animals for scientific research of Ahmadu Bello University Zaria.

Induction of Experimental Type 2 Diabetes Mellitus

Obesity was first induced by feeding the animals in groups 2-5 with high fat diet (100g animal fats mixed with 100g of livestock growers' mash for five (5) weeks. The animal fat was obtained from the renal pad of slaughtered cows. At the end of 5_{th} week the animals were fasted for 12 hours, but allowed water *ad-libitum* and a freshly prepared streptozotocin at a dose of 35mg/kg body weight in a citrate buffer with a P_H of 4.5 was intra-peritoneally injected (Iliya *et al.,* 2016). Diabetes was allowed to develop and stabilized in the STZ-treated rats over a period of 72 hours. Hyperglycaemia in the rats was confirmed by conducting a glucose tolerance test on the fasted rats over a 2 hours period with the aid of a glucometer (Accu-Check Active Roche®). At the end of the tolerance test the animals were given dextrose solution (2g glucose in 100ml distilled water) to prevent hypoglycemia. Rats that displayed a sustained rise of ≥200mg/dl in blood glucose level were confirmed to be diabetic (Iliya *et al*., 2017).

Experimental design

After successful diabetic induction, the animals were grouped and treated as follows:

Group 1 (normal control): Administered 1ml/kg distilled water

Group 2 (Diabetic control): Administered 1ml/kg distilled water

Group 3 (Diabetic-Metformin): Administered 50mg/kg metformin

Group 4 (Diabetic High Dose BCLE): Administered 800 mg/kg *Byrsocarpus coccineus* leaf extract

Group 5 (Diabetic Low Dose BCLE): Administered 400 mg/kg *Byrsocarpus coccineus* leaf extract

The LD_{50} of Akpan *et al*. (2012) was adopted in this study. All treatments were done by oral gavage for 21 days.

At the end of the duration of treatment the rats were anaesthesized with an injection of Ketamine Hydrochloride at a dose of 50mg/kg body weight intra-peritoneally. A vertical incision was made along the rat's abdominal wall. The thoracic diaphragm was

incised to gain entry into the thoracic cavity and viscera. A cardiac puncture was done and 1ml of blood was collected into plain tubes and centrifuged at 10 rad/sec for 7 minutes to obtain the serum, which was stored at -20$_o$C and later used to measure the serum glucose level in the rats.

Sample Collection and Sectioning

Thereafter normal saline was used to flush the rat body systems for a period of 30 minutes with after which normal saline was replaced with the neutral buffered formalin solution for another 30 minutes. At the end of the perfusion-fixation, the pancreas was carefully dissected out and subjected to preparation techniques for stereological estimation of pancreatic islet volumes and histological analysis.

Isotropic Uniform Random Sampling (IURS)

IURS was performed using the Orientator Grid. At first the pancreas was placed at (a) center of the circle with equal division and a random number (2) was calculated and selected from the random number table and the sample was cut. Secondly (b) each part of the cut sample was again placed on a second circle with unequal divisions and another random number (6) was selected and the sample were cut here (Ali *et al.*, 2012). The method is shown in figure 1 below.

After IURS, samples were processed using normal routine histological techniques (Bancroft, 2002). Tissues were embedded in molten paraffin wax in cassettes. Sections were cut using a Rotatory Microtome (Leica) at 10µ. A random number 5 was calculated and selected from the random number table and 10 sections were systematically picked from the ribbon of cut sections. Floated out in a warm bath, mounted on charged slides, air-dried and stained with Haematoxylin and Eosin. Photomicrographs were taken with a microscope digital camera at 510 mega-pixel (DCM ScopePhoto® China) and a light microscope (Leitz Wetzlar, Germany) at ×250 magnification.

Islet of Langerhans volume estimations

A test point counting grid (cavalieri estimator) was superimposed on the pancreatic tissue sections and single test points hitting the pancreatic islets were counted and summed.

The volume changes of the pancreatic islets were calculated as previously described by Gundersen *et al.* (2002) *using* the following imputations:

V (mm3) = $\bar{T}$x *A P*x ∑pi (where $\bar{T}$= distance from the 1$_{st}$ section to the 10$_{th}$ section; *A P* = area per point; ∑pi=sum of test points)

Noise due to errors in the sampling was calculated thus: noise= 0.0724 x B/√Ax √n x ∑pi

Variations due to the systematic random sampling of the serial sections was calculated: VARsurs=3(A-noise)-4(B+C) +C

Total variance (TVAR) = noise + VARsurs

Coefficient of error due to the entire sampling process (CE) was calculated: CE=√TVAR/∑pi.

Statistical Analysis

Results were expressed as Mean ± SEM. One-way ANOVA was used to test for statistical significant difference at $p<0.05$. Tukey post-hoc test was used to determine where the difference lies. All statistics was done using SPSS (version 20).

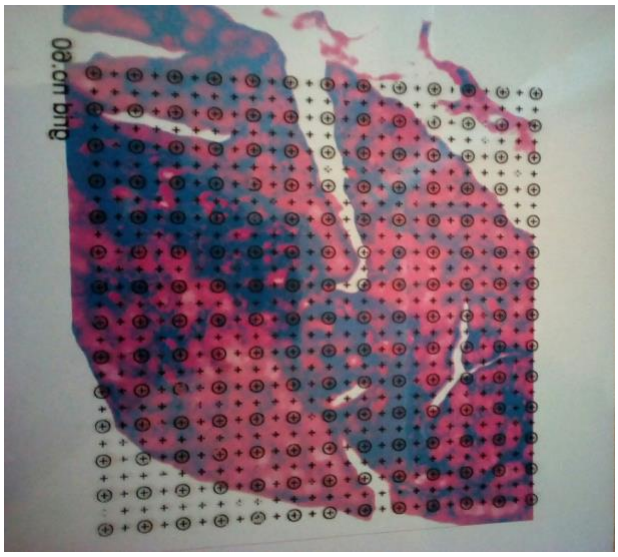

Plate 1:
The Cavalieri Estimator Grid used for the study.

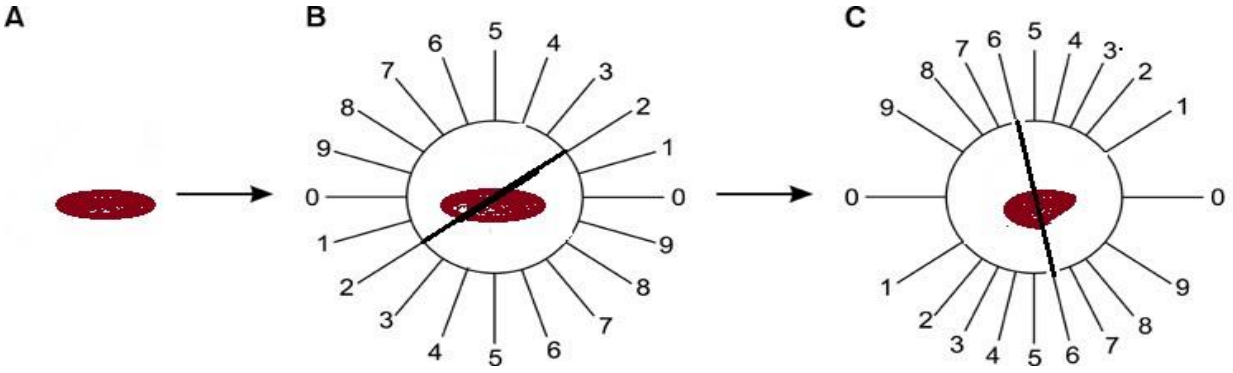

Figure 1: Isotropic Uniform Random Sampling (Ali et al., 2012)

RESULTS

Serum Blood Glucose Level:

Figure 2 shows the effect of *Byrsocarpus coccineus* leaves leaf extract (BCLE) on the blood glucose levels of the normal and diabetic rats. The result indicated that there was a significant increase ($p<0.05$) in the blood glucose level of the diabetic rats when compared to the normal control. Treatment interventions with standard drug as well as different dosages of BCLE significant decreased ($p<0.05$) the blood glucose level when compared to the diabetic control.

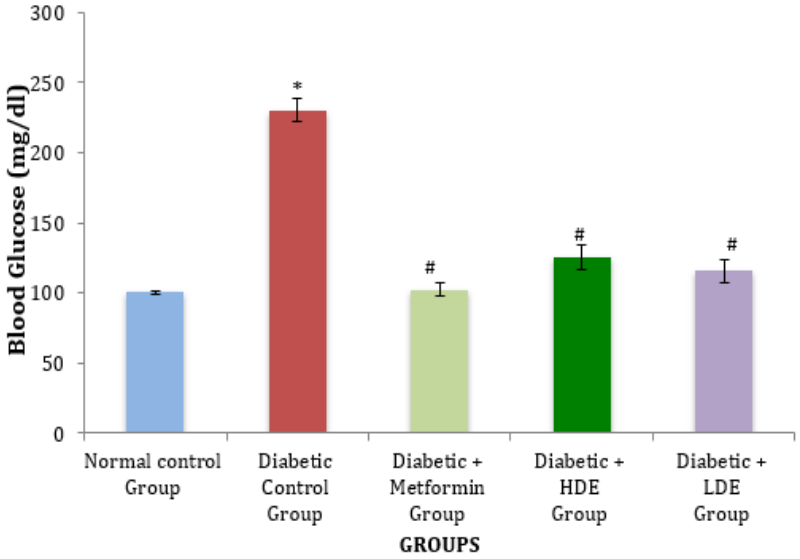

Figure 2.
Effect of *Byrsocarpus coccineus* Leave Extract on Serum Fasting Blood Glucose in High-fat Diet and STZ-induced Diabetic Rats.

Table 1:
Effect of *Byrsocarpus coccineus* Leave Extract on Volume Estimation of Pancreatic Islets in High-fat diet and STZ-induced Diabetic Rats

Groups	Volume mm3	Noise	VAR_{SURS}	Total Variance	Coefficient of Error	Mean ± SEM	P
1	22620	1317.52	–245243.56	–243926.04	0.65	75.4 ± 4.217	0.01
2	5220	149.55	–14745.65	–14596.11	0.69	12.2 ± 0.964*	
3	15990	812.11	–87533.03	–86720.93	0.69	53.3 ± 2.591	
4	11580	487.867	–68708.60	–68220.73	0.67	42.89 ± 2.176	
5	15540	688.97	–104966.91	–104277.94	0.62	51.8 ± 4.657	

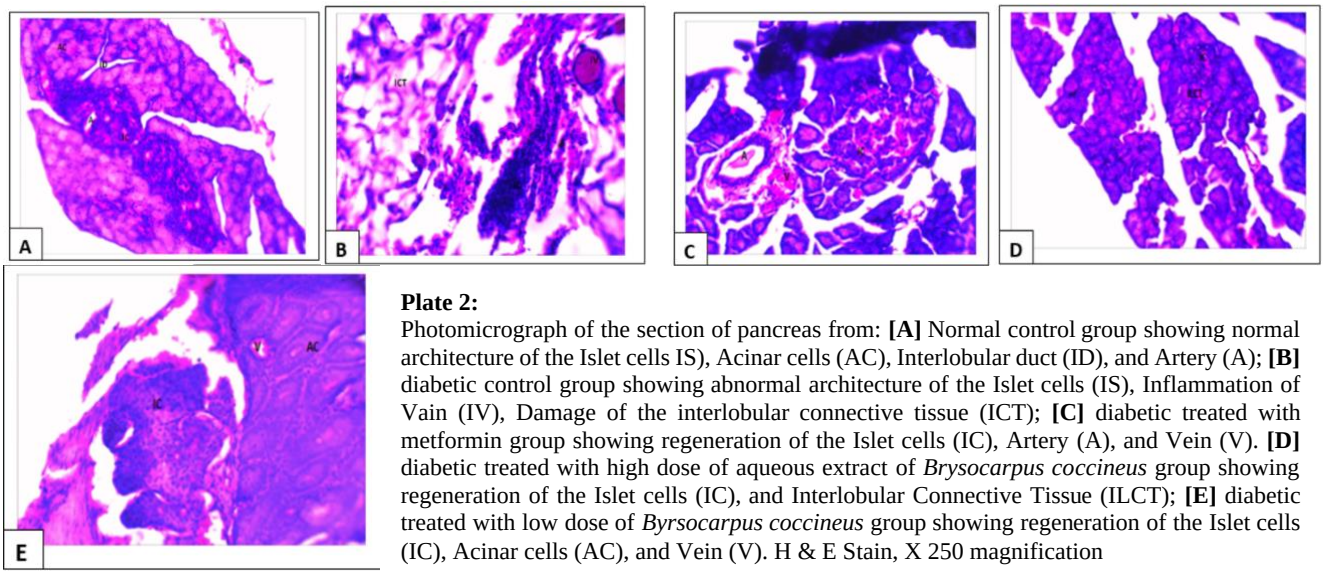

Plate 2:
Photomicrograph of the section of pancreas from: **[A]** Normal control group showing normal architecture of the Islet cells IS), Acinar cells (AC), Interlobular duct (ID), and Artery (A); **[B]** diabetic control group showing abnormal architecture of the Islet cells (IS), Inflammation of Vain (IV), Damage of the interlobular connective tissue (ICT); **[C]** diabetic treated with metformin group showing regeneration of the Islet cells (IC), Artery (A), and Vein (V). **[D]** diabetic treated with high dose of aqueous extract of *Brysocarpus coccineus* group showing regeneration of the Islet cells (IC), and Interlobular Connective Tissue (ILCT); **[E]** diabetic treated with low dose of *Byrsocarpus coccineus* group showing regeneration of the Islet cells (IC), Acinar cells (AC), and Vein (V). H & E Stain, X 250 magnification

Volume Estimation of pancreatic islets:

As shown in table 1, a significant decrease ($p<0.05$) in the estimated volume of pancreatic islets in diabetic control group when compared to normal control group. There was a significant increase ($p<0.05$) in the volume of pancreatic islets in the diabetic metformin group (group 3); high dose of BCLE at 800mg/m (group 4) and low dose of BCLE at 400mg/ml (group 5) when compared to diabetic control.

Histological Evaluation of Pancreatic Tissue

Plate 2 shows the result the histology of the pancreas. In plate 2a, the section of pancreas from normal control group shows normal architecture of the Islet cells (IS), Acinar cells (AC), Interlobular duct (ID), and Artery (A). Plate 2b, the diabetic control group revealed damaged pancreatic islet cells, the exocrine pancreatic cells (acinar cells and ductal cells), the interlobular duct, interlobular connective tissue, and blood vessels. But there was a gradual increase in the regeneration of the pancreatic islet cells, interlobular duct and connective tissue in the diabetic metformin group (plate 2c); high dose of BCLE at 800mg/m (Plate 2d) and low dose of BCLE at 400mg/ml (plate 2e)

DISCUSSION

Loss of pancreatic islet cell mass is associated with type 2 diabetes (T2D) (Bonner-Weir and O'Brien, 2018). The present study aimed at evaluating the volume of pancreatic islets and pancreatic pathological changes in type 2 diabetic rats following treatment with aqueous *Byrsocarpus coccineus* leaves extract (BCLE). Our findings showed that the aqueous extract BCLE attenuated the hyperglycemic conditions of the diabetic rats as was reported by Dada *et al.* (2013). The anti-hyperglycaemic potential of the extract observed in our study was better in the group treated with the lower dose (400 mg/kg body weight), which was not the case in respect to study by Dada *et al.* (2013). Nevertheless *Byrsocarpus coccineus* has been confirmed in this study to possess anti-hyperglycaemic activity. In line with the outcome of the glycemic control, the pancreatic islets volume estimation results showed that the aqueous extract of the leaves of *Byrsocarpus coccineus* elicited an increase in the volume of the pancreatic islets in the treated rats. The increase in the islet volumes was also slightly better at the lower dose of the extract. An increase in the total number of islet cells may reflect an increase in the total volume of the islets and may provide a clue to the improvement in the glycaemic control observed in the groups treated with the extract. Skau *et al.* (2001) had initially reported that an increase in islet volumes could be due to growth of existing islet cells or production of new ones from intra-islet stem/progenitor cells and β-cells are primary sources for these types of new cells. It can be deduce that the aqueous extract of *Byrsocarpus coccineus* leaves possibly contain biomolecules that has the potential to stimulate remnant of β-cells of the damaged pancreatic islets or influence a regeneration of the β-cells in these islets. With respect to the studies reported by Dada *et al*. (2013) that the high dose of extract *Byrsocarpus coccineus* increased insulin level, it can be might inferred that the reduced blood glucose levels observed in the extract treated group is a direct consequence of insulin action Also, Streptozotocin injections at low dose coupled with a high-fat food regimen like the one used in this study can expose an animal to type 2 diabetes mellitus with a selective destruction of the islet of Langerhans β-cells thus leading to a decrease in the blood insulin level and thus the poor glycaemic control observed in the diabetic control rats (Reed *et al*., 2000; Imam and Ismail, 2012).

In addition, the hyperglycemic condition and the decrease in the volumes of pancreatic islets of the rats in the diabetic control group was evident by the result of the histology that revealed an abnormal architecture of the Islet cells (IS), with Inflammation, and Damage of the interlobular connective tissue and Blood vessel.

It can be concluded that the aqueous extract of *Byrsocarpus coccineus* possess potent anti-diabetic efficacy in type 2 diabetic rats by restoring pancreatic damaged β-cells which carry a direct linear relationship with the total islet volumes thus the increases in the pancreatic islet volumes and attenuating the hyperglycaemic condition.

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Effects of *Uvaria chamae* Root Extracts on Blood Glucose, Inflammatory Markers, Lipid Profile, Liver and Renal Status in Streptozotocin–induced Diabetic Rats

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Summary: *Uvaria chamae* roots are traditionally used in the treatment of diabetes in many parts of the world, but the use of the extracts in the treatment of diabetes has not been scientifically validated. Thirty-six Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 ± 7.20g]. Diabetes mellitus was induced by a single administration of streptozotocin (60 mg/kg) intraperitoneally. Normal and diabetic rats were treated with aqueous or ethanolic extract (300 mg/kg body weight/day/rat) of *Uvaria chamae* for 35 days. Rats were allowed free access to food, and extract added to the water bottle. Animals were euthanized on day 35 after an overnight fast and blood was collected for glucose, renal function, liver, serum lipid profile, and inflammatory markers assays. The blood glucose levels decreased by 38% and 53% in the diabetic rats administered aqueous or ethanolic extract respectively compared to an increase in the diabetic control (45%). The levels of TC, TG, LDL-C, VLDL-C, TG/HDL-C, and non-HDL-C were decreased in untreated rats, while the HDL-C was increased when the extracts were administered. There was a diminishing trend in IL-6, TNF-α and IL-1β levels in the treated diabetic groups. Serum creatinine level was slightly elevated in the diabetic group administered ethanolic extract. Overall, the consumption of *Uvaria chamae* extracts lowered blood glucose levels, lipid profile and increased HDL-C, while the IL-6 was decreased. The non-significant changes in renal function parameters indicated no adverse effects on the kidney in this short-term study.

Keywords*:* Blood glucose, Diabetes, Inflammation, *Uvaria chamae*

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INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Sustained hyperglycemia is associated with the development of diabetic complications in patients with the disease (Makita *et al*., 1991). It is estimated that 552 million people will be diagnosed with the disease by 2030 (Whiting *et al*., 2011). Inflammatory cytokines are believed to exert central roles in the development of renal disease in diabetic patients (Navarro-Gon'zalez *et al*., 2011). Hyperglycemia can induce the expression of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), leading to the development of chronic subclinical inflammatory status in diabetes mellitus (Goldberg, 2009). Interestingly, IL-6 a crucial pro-inflammatory cytokine can promote insulin secretion at low concentration (Os'orio, 2015). A high level of IL-6 may damage β-cells and promote apoptosis (Oh *et al*., 2011). Interleukin-6 has also been reported to increase lipolysis in adipocytes as well as the release of free fatty acids that may affect mitochondria and glucose transporter 2 (GLUT-2) function and insulin sensitivity (Dessein *et al*., 2002). An increased IL-6 expression accompanies abnormal glucose metabolism (Huan *et al*., 2016). Hyperglycemia increases the generation of free radicals by auto-oxidation of glucose, and the increase in free radicals may lead to cell damage (Sharma *et al*., 2006). Alteration in serum lipid profile in people with diabetes has been reported to increase the risk of coronary heart disease (Massing *et al*., 2001). In the diabetic state, prolonged hyperglycemia may result in metabolic complications which will potentiate the release of reactive radical, that could interfere with the integrity of liver cells. For example, uncontrolled diabetes mellitus has been reported to be associated with elevated liver enzymes (Kim *et al*., 2006). There is currently no cure for diabetes mellitus, but there is an increasing demand for natural products with antidiabetic activities. Medicinal plants have been extensively used as an alternative medicine for the

management of diabetes mellitus (Mahalingam and Krishnan, 2008). *Uvaria chamae* is a medicinal plant found mostly in the tropical rain forest of West Africa. In Nigeria, it is used for the treatment of diabetes, diarrhea, hypertension, cough, hemorrhoids, kidney and bladder diseases (Hufford and Lasswell, 1976). It is commonly known as finger root or bush banana and belongs to the family *Annonaceae*. When ripe, the fruits are yellow and have a sweet pulp that is widely eaten. All parts of the plant are fragrant with widespread medicinal use in West Africa (Omajali *et al*., 2011). However, there is a shortage of scientific evidence to validate the beneficial role of the plant extract in the management of diabetes mellitus. In this study, the effects of the aqueous or ethanolic extract of the root on blood glucose, inflammatory cytokines, lipid profile, liver and renal functions in normal and diabetic rats were evaluated.

MATERIALS AND METHODS

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Sustained hyperglycemia is associated with the development of diabetic complications in patients with the disease (Makita *et al*., 1991). It is estimated that 552 million people will be diagnosed with the disease by 2030 (Whiting *et al*., 2011). Inflammatory cytokines are believed to exert central roles in the development of renal disease in diabetic patients (Navarro-Gon'zalez *et al*., 2011). Hyperglycemia can induce the expression of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), leading to the development of chronic subclinical inflammatory status in diabetes mellitus (Goldberg, 2009). Interestingly, IL-6 a crucial pro-inflammatory cytokine can promote insulin secretion at low concentration (Os'orio, 2015). A high level of IL-6 may damage β-cells and promote apoptosis (Oh *et al*., 2011). Interleukin-6 has also been reported to increase lipolysis in adipocytes as well as the release of free fatty acids that may affect mitochondria and glucose transporter 2 (GLUT-2) function and insulin sensitivity (Dessein *et al*., 2002). An increased IL-6 expression accompanies abnormal glucose metabolism (Huan *et al*., 2016). Hyperglycemia increases the generation of free radicals by auto-oxidation of glucose, and the increase in free radicals may lead to cell damage (Sharma *et al*., 2006). Alteration in serum lipid profile in people with diabetes has been reported to increase the risk of coronary heart disease (Massing *et al*., 2001). In the diabetic state, prolonged hyperglycemia may result in metabolic complications which will potentiate the release of reactive radical, that could interfere with the integrity of liver cells. For example, uncontrolled diabetes mellitus has been reported to be associated with elevated liver enzymes (Kim *et al*., 2006). There is currently no cure for diabetes mellitus, but there is an increasing demand for natural products with antidiabetic activities. Medicinal plants have been extensively used as an alternative medicine for the management of diabetes mellitus (Mahalingam and Krishnan, 2008). *Uvaria chamae* is a medicinal plant found mostly in the tropical rain forest of West Africa. In Nigeria, it is used for the treatment of diabetes, diarrhea, hypertension, cough, hemorrhoids, kidney and bladder diseases (Hufford and Lasswell, 1976). It is commonly known as finger root or bush banana and belongs to the family *Annonaceae*. When ripe, the fruits are yellow and have a sweet pulp that is widely eaten. All parts of the plant are fragrant with widespread medicinal use in West Africa (Omajali *et al*., 2011). However, there is a shortage of scientific evidence to validate the beneficial role of the plant extract in the management of diabetes mellitus. In this study, the effects of the aqueous or ethanolic extract of the root on blood glucose, inflammatory cytokines, lipid profile, liver and renal functions in normal and diabetic rats were evaluated.

RESULTS

Biochemical Assessment

There were no significant changes ($p > 0.05$) in the food consumed (Table 1) and body weight of the rats in this study (Table 2). There were decreases of 38% and 53% in fasting blood glucose in the diabetic rats administered aqueous or ethanolic extract respectively compared to the increase in the diabetic control (45%) group. The administration of aqueous or ethanolic extract in normal rats resulted in slight decreases in fasting blood glucose of 0.2% and 4% respectively compared to the increase in normal control (11%) group. This study showed that BUN was significantly ($p<0.05$) increased in the diabetic control compared to normal control. In contrast, serum creatinine was non-significantly increased in the diabetic group administered ethanolic extract in comparison to normal control (Table 3).

The administration of aqueous or ethanolic extract to the normal and diabetic rats showed a decrease in total TC, TG, TG/HDL-C ratio, LDL-C, VLDL-C and non-HDL-C when compared to their respective controls

Table 1:
Food intake (g/week) by normal and diabetic rats administered aqueous or ethanlic extract of *Uvaria chamae*.

Group	Food Intake (g)
Normal Control	248.26 ± 44.22
Normal + Aq. Extract	249.27 ± 26.21
Normal + Eth. Extract	243.29 ± 43.13
Diabetic Control	405.55 ± 27.90
Diabetic + Aq. Extract	303.74 ± 74.31
Diabetic + Eth. Extract	381.29 ± 10.64

Values are means ± SEM of 4-6 determinations, and they were not significantly (p > 0.05) different among the groups. Eth= Ethanolic. Aq= Aqueous.

Table 2: Rats weight (g) for the duration (35 days) of study

Group	Initial Weight (g)	Final Weight (g)	Weight Change (%)
Normal Control	264.64 ± 24.01	314.12 ± 43.03	18.70
Normal + Aq. Extract	261.16 ± 16.90	306.33 ± 31.01	17.32
Normal + Eth. Extract	256.88 ± 18.70	287.48 ± 27.00	11.91
Diabetic Control	252.75 ± 25.11	235.07 ± 31.51	-6.90
Diabetic + Aq. Extract	274.58 ± 18.92	265.63 ± 31.30	-3.30
Diabetic + Eth. Extract	301.75 ± 3.41	247.00 ± 6.62	-18.11

Values are mean ± SEM of 4-6 determinations, and they were not significantly ($p > 0.05$) different among the groups. Eth= Ethanolic. Aq= Aqueous.

Table 3: Kidney function parameters (uric acid, BUN and creatinine) in the serum of normal and diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae.*

Group	Uric Acid (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)
Normal Control	7.38 ± 1.01	28.66 ± 2.22	1.27 ± 0.02
Normal + Aq. Ext	12.41 ± 2.02	30.50 ± 5.40	1.25 ± 0.11
Normal + Eth. Ext	10.21 ± 2.50	32.00 ± 1.91	1.48 ± 0.10
Diabetic Control	7.05 ± 0.21	70.87 ± 6.62	1.82 ± 0.21
Diabetic + Aq. Ext	6.75 ± 0.71	70.75 ± 5.00*	1.68 ± 0.20
Diabetic + Eth. Ext	5.98 ± 0.20	77.87 ± 8.02*	1.92 ± 0.40

Values are some indices of renal function in mg/dl and are expressed as mean ± SEM.
*Vertical * denotes significant differences ($p < 0.05$) from normal control (Duncan Multiple Range Test). BUN = Blood Urea Nitrogen, Aq. Ext = Aqueous Extract, Eth. Ext = Ethanolic Extract*

Table 4: Blood lipid profile in Normal and Diabetic rats treated with *Uvaria chamae* extract.

Group	TC (mg/dL)	TG (mg/dL)	HDL-C(mg/dL)	TG/HDL-C	LDL-C (mg/dL)	VLDL-C (mg/dL)	Non-HDL-C (mg/dL)
Normal Control	117.26 ±1.70	80.39 ± 4.81	3.10 ± 0.31	38.81 ± 5.42	90.40±3.82	17.85±1.21	108.25±3.81
Normal + Aq. Ext	89.64 ± 2.31	48.23 ± 4.72	3.24 ± 0.32	22.07 ± 2.81	82.05±3.61	11.11±1.60	93.17±4.40
Normal + Eth. Ext	81.45 ± 6.52*****	36.94 ± 4.63	4.82 ± 0.31	72 ± 0.99*****	79.94±5.30	8.37±1.21	81.98 ± 6.11
Diabetic Control	133.16 ± 9.92	204.87±44.90	8.18 ± 0.60	26.70 ± 5.51	94.40±13.3	35.54±8.31	125.10±9.10
Diabetic + Aq. Ext	104.09 ±8.70	165.78 ±58.82	7.25 ± 0.41	14.83 ± 2.41	74.76±3.01	26.22±6.13	108.10±9.91
Diabetic + Eth. Ext	107.41±18.41	121.59 ± 27.90	11.28±0.40#	12.00 ± 0.30	70.61±7.40	20.86±4.70	98.57±15.50

*Values are serum concentrations of lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C and non-HDL-C) in mg/dl and are expressed as mean ± SEM. Vertical * denotes significant differences ($p < 0.05$) from normal control, whereas # denotes significant differences ($p < 0.05$) in diabetic groups treated with Uvaria chamae extract when compared to diabetic control (Duncan Multiple Range Test). Aq. Ext= Aqueous Extract. Eth. Ext= Ethanolic Extract.*

(Table 4). The HDL-C was significantly ($p < 0.05$) increased in the diabetic group administered ethanolic extract when compared to diabetic control. There was a significant decrease in the concentration of ALT and ALP in the diabetic groups administered ethanolic and aqueous extracts when compared to their respective controls (Table 5). There was a significant ($p<0.05$) decrease in IL-6 and TNF-α in the Diabetic + Aq. Ext group compared to the Diabetic Control. While in the Diabetic + Eth. Ext, the IL-1β was significantly ($p<0.05$) reduced with a corresponding decrease in IL-6 and TNF-α when compared to the diabetic control (Table 6)

Histopathological findings in pancreas (figure 1) showed exuberant islet [A] and exocrine glands [B] in the pancreas of rats treated with aqueous or ethanolic extracts [plates 2 and 3] when compared to the normal control [plate1]. There was vascular congestion [A], vascular wall thickening and luminal narrowing [B] and mild infiltrates of chronic inflammatory cells in the pancreas of untreated diabetic rats [plate 4]. The pancreas of diabetic rats treated with aqueous or ethanolic root extract of *Uvaria chamae* showed resurgence of islet cells [A] and mild vascular congestion [B] [plates 5 and 6]. However, there was a mild ductal protein deposit [C] in plate 6. (H&E x 400).

The liver of rats treated with aqueous or ethanolic extract at 300 mg/kg body weight (figure 2) showed mild vascular congestion [A] plate 8, portal congestion [A] and moderate Kupffer cells [B] activation [plate 9] when compared to normal control [plate7]. . There were vascular congestion and dilatations [A] in the diabetic group treated with aqueous extract [plate 11], and vascular congestion [A] and moderate Kupffer cell activation [B] in the diabetic rats administered ethanolic extract [plate12] when compared to the diabetic control [plate 10]. (H&E x 400)

Table 5:
Liver function test (ALP, ALT, AST) in the serum of normal and streptozotocin- induced diabetic rats

Group	ALP(U/L)	ALT(U/L)	AST(U/L)
Normal Control	34.55 ± 7.06	43.94 ± 1.32	2.32 ± 0.52
Normal + Aq. Ext	28.79 ± 3.31	11.64 ± 1.31*	1.52 ± 0.21
Normal + Eth. Ext	34.20 ± 4.20	1.96 ± 0.21*	3.27 ± 0.80
Diabetic Control	188.87 ± 32.21	15.71 ± 8.70	3.49 ± 1.71
Diabetic + Aq. Ext	84.26 ± 23.01 #	17.46 ± 4.80	3.49 ± 1.02
Diabetic + Eth. Ext	96.74 ± 13.82 #	2.91 ± 0.21#	1.74 ± 0.01

*Values are means ± SEM of 4-6 determinations. Vertical * denotes significant difference ($p < 0.05$) from normal control, whereas # denotes significant difference ($p < 0.05$) in diabetic groups treated with Uvaria chamae extract when compared to diabetic control. Aq. Ext = Aqueous Extract, Eth. Ext = Ethanolic Extract.*

Table 6: Inflammatory markers (IL-6, IL-1β and TNF-α) in serum of normal and Streptozotocin- induced diabetic rats.

Group	IL-6 (pg/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)
Normal Control	156.85 ± 11.01	113.75 ± 43.82	121.67 ± 19.50
Normal + Aq. Extract	152.17 ± 13.20	203.85 ± 14.81	360.03 ± 27.41
Normal + Eth. Extract	194.51± 38.61	183.15 ± 3.61	242.38 ± 10.42
Diabetic Control	316.99 ± 4.51	592.27 ± 25.50	725.09 ± 75.10
Diabetic + Aq. Extract	84.04 ± 16.50#	471.72 ± 76.20	149.01 ± 11.30 #
Diabetic + Eth. Extract	222.13 ± 33.10	201.42 ± 32.91#	503.66 ± 39.11

Values are means ± SEM of 4-6 determinations. Vertical #denotes significant differences ($p < 0.05$) in diabetic groups treated with Uvaria chamae extract when compared to diabetic control (Duncan Multiple Range Test). Eth= Ethanolic. Aq= Aqueous. IL = Interleukin, TNF-α = Tumor Necrosis Factor Alpha.

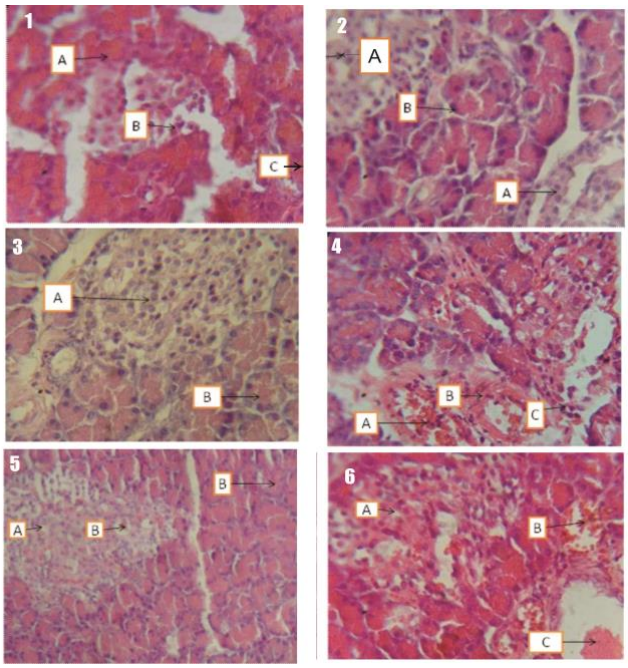

Figure 1.
Histopathologically findings in pancreas. Control pancreas [plate 1] showed exocrine glands [A], islets of Langerhans [B] and interlobular ducts. The pancreas of rats treated with 300 mg/kg aqueous extract [plate 2] showed exuberant islet [A] and exocrine glands [B]. Plate 3 is pancreas that was treated with 300 mg/kg of ethanolic extract showing exuberant islet [A] and exocrine glands [B]. The diabetic control pancreas [plate 4] showed severe vascular congestion [A], vascular wall thickening and luminal narrowing [B] and mild infiltrates of chronic inflammatory cells [C]. Plate 5 is the diabetic pancreas treated with 300 mg/kg aqueous extract showing resurgent islet [A] and mild vascular congestion [B]. While, plate 6 is the diabetic pancreas treated with 300 mg/kg ethanolic extract and showed resurgent islets [A], moderate vascular congestion [B] and mild ductal protein deposit [C]. (H&E x 400)

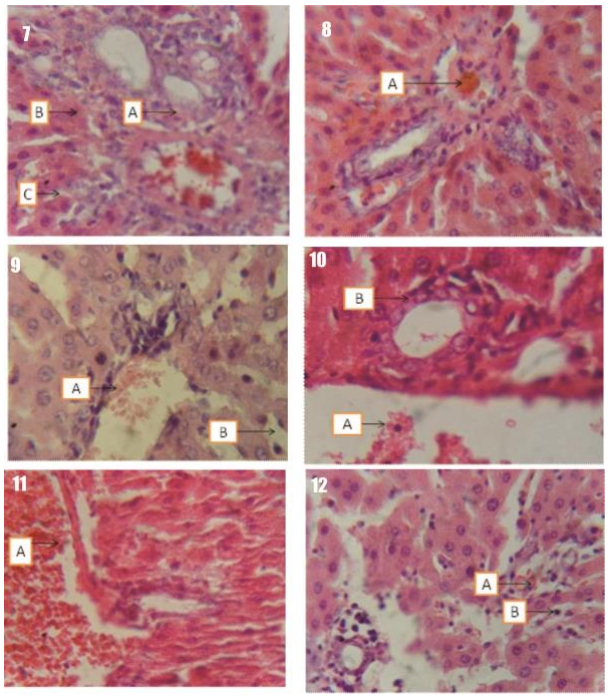

Figure 2.
Histopathological findings in liver. Control liver [plate 7] showed bile duct [A], hepatocyte [B] and sinusoids [C]. The liver of rats treated with 300 mg/kg aqueous extract [plate 8] showed mild vascular congestion [A]. The liver of rats treated with 300 mg/kg of ethanolic extract [plate 9] showed portal congestion [A] and moderate Kupffer cells activation [B]. Plate 10 is the liver of diabetic control rats which revealed portal congestion and dilation [A], and periportal infiltrates of chronic inflammatory cells [B]. In the diabetic liver treated with 300 mg/kg of aqueous extract [plate11] showed vascular congestion and dilation [A], while plate 12 is diabetic liver treated with 300 mg/kg of ethanolic extract showing vascular congestion [A] and moderate Kupffer cell activation [B]. (H&E x 400)

DISCUSSION

Streptozotocin has been an agent of choice to induce experimental diabetes mellitus due to its ability to cause specific necrosis of the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin (Bastaki, 2005). In the present study, the intraperitoneal injection of streptozotocin resulted in the significant increase in the blood glucose levels. Andrade-Cetto *et al*., 2019 reported the hypoglycemic effects of *Croton guatemalensis, Solanum americanum Mill and Neurolaena lobate*. While, Karunanayake *et al*., 1984 also reported the hypoglycemic effect of aqueous decoction of *Aegle marmelos* root bark in normal fasted rats. In this study, the administration of the aqueous or ethanolic extract of *Uvaria chamae* to the diabetic animals significantly decreased blood glucose which supports the traditional use of the extracts in the management of the disease. Diabetic control rats lost body weight when compared to the diabetic groups treated with the aqueous or ethanolic extract of *Uvaria chamae*. The decrease in body weight in the untreated diabetic rats may be attributable to gluconeogenesis with the associated increase in muscle wasting and loss of proteins in tissues (Shirwaikar *et al*., 2006). The observed increase in body weight of the treated diabetic rats may be a reflection of the ability of *Uvaria chamae* to promote cellular utilization of glucose with a subsequent decrease in blood glucose levels. The observed increasing trend in food intake in the diabetic control rats may be due to their inability to metabolize glucose for energy generation.

Abnormalities in kidney function progress by an alteration in hemodynamics that may lead to proteinuria, glomerulosclerosis and renal dysfunction (Zafar and Naqvi, 2010). The ability to overcome renal haemodynamic abnormality and the reduction of proteinuria is essential in preventing the decline of kidney function. Protein and nucleic acid metabolism result in the formation of non-protein nitrogenous compounds (Firdous *et al*., 2013). A significant elevation in serum creatinine, uric acid, and BUN levels is indicative of impaired renal function in people with diabetes (Mustafa *et al*., 2012). In oxidative stress, uric acid preserves the ability of vascular dilatation of the endothelium and prevents alteration of endothelial enzyme levels (Palsamy and Subramanian, 2008). The observed non-significant increase in BUN and creatinine, and lowered serum uric acid levels in diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* is an indication that the kidney function parameters may not be adversely affected in short-term use. However, long-term usage of the extracts should be done with caution as the extracts may adversely renal function. It has been reported that the conversion of streptozotocin to metabolites in the liver can result in catalytic membrane phospholipid peroxidation which ultimately reduces lipid export from the liver cells (Kumar *et al.*, 2007). The observed increase in TC, TG, LDL-C, and VLDL-C in the diabetic control rats may be associated with streptozotocin administration, which could be due to portal congestion, dilatation and periportal infiltrates of chronic inflammatory cells as observed histopathologically. Sphepherd (2005), reported that reduced HDL-C level in diabetic control rats is associated with insufficiency in fatty acid metabolism, and consequently result in hypercholesterolemia and hypertriglyceridemia which are standard features of lipid abnormalities in diabetes. Non- HDL cholesterol has been shown to be a predictor of cardiovascular risk (Virani, 2011), and contains cholesterol of all atherogenic particles (Grundy, 2002). A high TG/HDL-C ratio has been reported to be associated with several metabolic derangements like insulin resistance (Gonzalez-Gonzales-Chavez *et al*., 2011), beta cell dysfunction (Maturu *et al*., 2015) and diabetes incidence (Vega *et al*., 2014). In this study, we noted the enhancement of lipid profile constituents in the diabetic rats treated with aqueous or ethanolic extract of *Uvaria chamae*. Elevated liver enzymes are associated with diabetes mellitus (Kim *et al*., 2006), and prolonged hyperglycemia may result in metabolic complications through the release of reactive radicals that adversely alter the integrity of liver cells. The observed increase in ALP in the diabetic control may be associated with the cellular interaction of streptozotocin in the hepatocytes. Other workers have reported some liver pathology such as infiltration of nonspecific inflammatory cells which supports liver tissue damage in diabetic control seen in this study (Kumar *et al*., 2013; Khattab *et al*., 2013). However, the levels of ALP were significantly reduced by the extracts. We also noted a significant decrease in ALT activity in the diabetic rats treated with the ethanolic extract. However, the ethanolic extract was more effective in ameliorating the adverse effects of streptozotocin metabolites in the liver.

The observed elevated IL-6 expression positively correlated with glucose toxicity in the diabetic control group. However, there was a significant ($p<0.05$) reduction in IL-6 in the diabetic group administered aqueous, and a non-significant decrease in the diabetic group administered the ethanolic extract of *Uvaria chamae* when compared to the diabetic control. Similarly, the level of IL-1β in the diabetic group treated with ethanolic extract was significantly ($p<0.05$) reduced when compared to the diabetic control. Recently, attention has been focused on IL-1β, which is one of the primary pro-inflammatory cytokines that has been shown to cause tissue damage and organ failure. Thus, it is a crucial mediator in auto-inflammatory conditions (Dinarello, 2009; Dinarello *et al*., 2012). However, IL-1β is a vital role in Type 1 diabetes mellitus and has long been known to cause

pancreatic β-cell dysfunction and death (Mandrup-Poulsen *et al*., 2010). Interleukin-1β is produced and released by several cell types in response to tissue insult, or the case of diabetes mellitus, by pancreatic β-cells under hyperglycemic conditions (Maedler *et al*., 2002). Once present in the pancreatic environment it can act locally to inhibit insulin synthesis and secretion and induce pancreatic β-cell apoptosis. Hence, it is a promising target for diabetes therapeutic intervention (Mandrup-Poulsen *et al*., 2010). The administration of the aqueous or ethanolic extract of *Uvaria chamae* stimulates insulin release from the pancreas and prevent glucotoxicity in the microenvironment of β-cells of pancreas, thereby preventing its death as seen in the resurgence of islet cells. It has been reported that TNF-α is a possible mediator of insulin resistance and diabetes mellitus since it inhibits insulin signaling and impairs its secretion (Brunetti *et al*., 2014). Increased plasma level of pro-inflammatory cytokine, TNF-α is associated with chronic disease in Type I diabetes (Domingueti *et al*., 2016). Tumor necrosis factor stimulates hepatic lipogenesis, increases VLDL production, and raises serum lipid levels in diabetics (Feingold *et al*., 1990). Advanced glycosylation products have been shown to stimulate the production of TNF-α by macrophages (Vlassara *et al*., 1988). The data from this study showed a significant ($p<0.05$) reduction in the level of TNF-α in the diabetic group administered aqueous extract of *Uvaria chamae* in comparison to the untreated diabetic animals. Hence, the extract may have the ability to enhance insulin signaling and secretion from the β-cells of the pancreas, thereby preventing the complications associated with inflammatory conditions in diabetes mellitus. Histologically, the resurgence of islets of Langerhans in the diabetic rats administered aqueous or ethanolic extract when compared to diabetic control is supported by the significant ($p<0.05$) decrease in blood glucose observed in this study.

Overall, the consumption of aqueous or ethanolic extract of *Uvaria chamae* lowered blood glucose levels, lipid profile, and upregulation of the HDL-C which may be beneficial in the management of diabetes mellitus. The extracts administration especially the aqueous extract reduced the inflammatory cytokine (IL-6) that is commonly up-regulated in diabetes. Although the treatments in this short-term study did not significantly alter renal function, but the long-term use of the extracts should be done with caution.

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Cardiorenal Effects of Pharmaceutical Plant Effluent in Mice (*Mus musculus*)

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Summary: Many pharmaceutical industries carelessly handle their effluents and indiscriminately release same to aquatic environment. These effluents often find their ways into surface and ground waters, contaminating public water and thus, serving as a potential threat to animals and human health. In this study, we investigated the cardiorenal effects of chronic oral exposure to pharmaceutical effluent in mice. Thirty male mice (*Mus musculus*) were randomly divided into groups A-F and treated with 0.2 mLs 0.0 %, 2.5 %, 5.0%, 10.0%, 20.0% and 40% concentration (v/v, effluent/distilled water) of the effluent for 28 days, respectively. At the end of the experiment, the animals were sacrificed by cervical dislocation. Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined in serum and heart homogenate, while uric acid, creatinine and electrolytes (sodium, potassium, bicarbonate and chloride ions) were determined in serum only. Data were expressed as Means ± standard error of mean and values were considered significant at $p < 0.05$. Results showed that, oral exposure to pharmaceutical effluent reduced ($p < 0.05$) cardiac ALP, AST and ALT activities as well as serum ALT activity. However, serum activities of ALP, creatinine and uric acid were elevated ($p < 0.05$). Similarly, there was derangement of electrolytes (potassium, chloride, bicarbonate and sodium ions) in the exposed mice, compared with control. This study has demonstrated that poorly treated pharmaceutical effluent disrupted cardiac and serum enzyme activities, caused electrolytes imbalance and elevated serum uric acid level, suggesting that, drinking water contaminated with pharmaceutical effluent may impair kidney and cardiac functions. Further study, investigating the histology of the kidney and heart of the pharmaceutical effluent-exposed animals as well as mechanism(s) of cardiorenal toxicity of the effluent, should be carried out to exploit its roles in pathogenesis of cardiorenal diseases.

Keywords*:* cardiorenal, pharmaceutical effluent; electrolytes; enzymes; *Mus musculus*

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INTRODUCTION

Extensive industrialization, due to increasing population density and high urbanized societies, has resulted to global waste water management problem (Akpor et al., 2014). Many industries indiscriminately discharge contaminated and untreated waste products in to aquatic environment, causing pollution (Abu, 2012; Oyeniyi and Latunji, 2012). Inlands and estuary waters are both vulnerable to pollutants from industrial effluent discharge, affecting quality of water supply for domestic use (Osibanjo et al., 2011). This, therefore, causes global decrease in quality of drinking water below WHO standard and accounts for 3.1% death increase, globally (Khan et al., 2013; Pawari et al., 2015). It has also been reported that, heavy metals in water pollutants induce formation of reactive oxygen species (ROS), leading to organ damage in animals (Bando et al., 2005; Adegbesan and Adenuga, 2007).

Pharmaceuticals are among the important pollutants commonly released into environment (Stackelberg et al., 2004). Pharmaceutical industries generally produce large quantity of wastes during manufacturing and maintenance operations, which are subsequently released into water bodies (Chander et al., 2014). These wastes often consist of toxic biodegradable and non-biodegradable substances, and therefore remain public health concern. Although, concentration of pharmaceuticals discharged into environment is drastically reduced by various physical and biological processes occurring in aquatic ecosystem, trace concentrations of human and veterinary pharmaceutical compounds as well as their metabolites have been detected in water bodies like surface, ground and drinking waters (Kolpin et al., 2002; Benotti and Brownawell, 2009; Bruce et al., 2010). Exposure to trace amount of pharmaceuticals for a long time can result in considerable adverse effects, such as tissue damages and inhibition of cell proliferation, in humans and aquatic life (Brooks et al., 2003; Pomati et al., 2006).

In Nigeria, there is continuous increase in pharmaceutical industries, mostly located in riverside

areas. The discharge practices of these industries are too crude, putting society in danger, especially in the industrialized parts of the country (Ijeoma and Achi, 2011). The industries carelessly handle their wastes and indiscriminately discharge their effluents into water bodies, promoting aquatic pollution and affecting human and environmental health (Osaigbovo and Orhue, 2006; Idris et al., 2013). This is largely due to their ignorance of harmful effects of xenobiotics and microbes present in the effluent on both aquatic and terrestrial life as well as poor enforcement of stringent regulations prohibiting illegal discharge of effluents (Lateef et al., 2007; Adeoye et al., 2015).

Previous studies have revealed the mutagenicity, genotoxicity and hepatotoxicity of pharmaceutical effluent (Zhao *et al.*, 2007; Akintonwa *et al.*, 2009; Bakare *et al.*, 2009; Adeoye *et al.*, 2015) but there is paucity of information on its cardio-renal toxicity. Recently, we showed that oral exposure to pharmaceutical effluent impairs cardiac Na^+/K^+-ATPase activity and reduced cardiac weight index in mice (Abdulkareem et al., 2019). In this present study, we evaluated the effects of chronic oral exposure to pharmaceutical effluent on both renal and cardiac functions in mice.

MATERIALS AND METHODS

Effluent collection: The raw effluent from a pharmaceutical plant (which produces analgesics, anti-malarias, anesthetics, multivitamins and antibiotics) in Ilorin, Kwara State, Nigeria was collected in a 5L transparent plastic container (from the point of discharge into the environment). The collected effluent was filtered; the pH was taken and it was stored at 4°C until use.

Physico-chemical properties and heavy metal analysis: The effluent was analyzed for a number of standard physico-chemical properties, including: chemical oxygen demand (COD), total dissolved solids (TDS), alkalinity, biochemical oxygen demand (BOD), chlorides, nitrates, ammonia, and phosphates, according to methods described by APHA (1998). Eight metals which include: cadmium (Cd), chromium (Cr), iron (Fe), zinc (Zn), nickel (Ni), manganese (Mn), copper (Cu), and lead (Pb) were analyzed in the effluent sample according to standard analytical methods as previously reported (Bakare et al., 2009). Concentration of the metals were estimated by using an Atomic Absorption Spectrophotometer (Perkin Eelmer E., Analyst, 2000, USA).

Animals and experimental design: Thirty male mice (*Mus musculus)* of 8-10 weeks old were obtained from Central Research Laboratory, University of Ilorin, Ilorin, Nigeria. They were kept in a clean, frequently disinfected and well-ventilated animal house of the Department of Zoology, University of Ilorin, for 2 weeks in order to acclimatize. All mice were maintained under standard condition (12h light: 12h dark) and were exposed to standard feed and drinking water *ad libitum.* Handling of animals was kept in accordance with the regulation of University of Ilorin Ethical committee and in conformity to the NIH Guidelines on the care and use of laboratory animals. The mice were divided into 6 groups. Group A (control) mice received 0.2ml distilled water, while groups B-F were treated with 0.2 mLs 2.5%, 5.0%, 10.0%, 20.0% and 40% concentration (v/v, effluent/distilled water) of the effluent, respectively. All the treatments were administered orally and lasted for 28 days.

Tissue preparation: On 29_{th} post-treatment day, the animals were sacrificed by cervical dislocation. The heart was quickly excised, cleared of connective tissues, and transferred into the ice-cold 0.25M sucrose solution. The heart was homogenized in ice-cold 0.25M sucrose solution (1:5, w=v) as previously reported (Olatunji et al., 2006). The resulting homogenates were kept frozen overnight and centrifuged before use.

Enzymes assays: Method of Reitman and Frankel's (1957) was employed in estimating alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Activity of alkaline phosphatase (ALP) was determined, using Babson et al. (1966) method.

Determination of serum uric acid and creatinine concentrations: Concentrations of serum uric acid and creatinine were determined, using the method of Tietz (1994) as outlined in Randox kits, UK.

Determination of serum electrolytes: Potassium, sodium and chloride were determined by the method of Tietz *et al.* (1996), while serum bicarbonate level was assessed by the method of Roth and Chan (2001).

Statistical analysis

All data were expressed as means ± standard error of mean (SEM). Statistical analysis was performed with Graphpad Prism 5 (GraphPad Software, USA). Mean values of variables among the groups was compared by One-way analysis of variance (ANOVA), following Bonferroni Post-*hoc* test. Values were considered significant at $p < 0.05$.

RESULTS

Physicochemical properties of the raw pharmaceutical effluent: Details of the physicochemical properties of the effluent have been provided in our previous study (Abdulkareem et al., 2019). The pH value (6.40) of the effluent falls within national permissible limit (NESREA). Concentrations

of Cadmiun (Cd), Chromium (Cr), Zinc (Zn), and Copper (Cu), phosphate, alkalinity and TDS were lower than international (USEPA) and national (NESREA) recommended limits, while iron (Fe), manganese (Mn) and ammonia (NH_3) in the sample were higher than the limits. Lead (Pb) and nickel (Ni) were below detectable limits.

Cardiac and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP): As shown in figures 1, 2 and 3), analyses of enzyme activities showed that, 28 day oral administration of pharmaceutical effluent significantly decreased ($p < 0.05$) cardiac activities of ALP, AST and ALT. Similarly, there was a significant decrease ($p < 0.05$) in serum activity of ALT in the exposed mice. In contrast, there was an increase ($p < 0.05$) in serum activity of ALP while serum activity of AST was not significantly changed ($p > 0.05$), when compared with control.

Serum creatinine and uric acid: Figure 4 shows that, chronic oral exposure to pharmaceutical effluent at 5 %, 10 %, 20 % and 40 % concentrations significantly ($p < 0.05$) elevated serum uric acid and creatinine levels, as compared with control.

Serum electrolytes: There was a significant ($p < 0.05$) concentration independent increase in serum potassium, chloride and bicarbonate ions values, meanwhile values of sodium ion decreased (concentration independent) significantly ($p < 0.05$) as compared with control (Table 1).

Fig. 1: Effect of pharmaceutical plant effluent on cardiac and serum alkaline phosphatase (ALP) activities in mice. Oral exposure to the effluent significantly decreased cardiac ALP activity, but increased the activity in the serum (*$p < 0.05$ vs control).

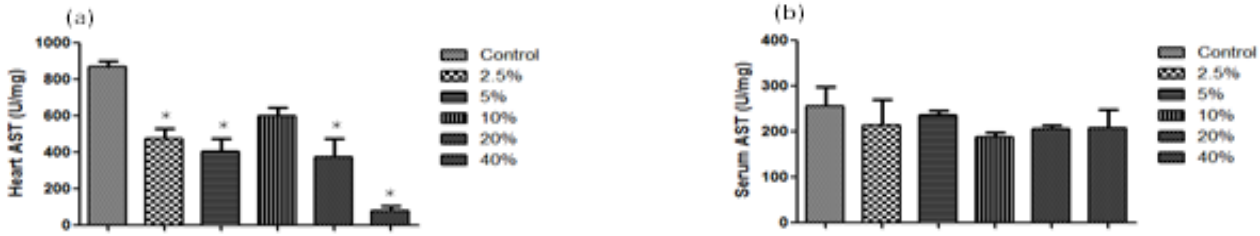

Fig. 2: Effect of pharmaceutical plant effluent on cardiac and serum aspartate aminotransferase (AST) activities in mice. Oral exposure to the effluent significantly decreased cardiac AST activity, whereas, there was no significant increase in serum activity of the enzyme (*$p < 0.05$ vs control).

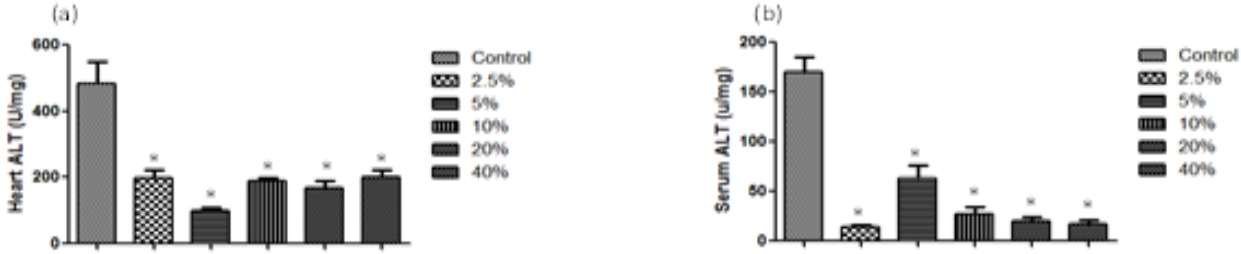

Fig. 3: Effect of pharmaceutical plant effluent on cardiac and serum alanine aminotransferase (ALT) activities in mice. Oral exposure to the effluent significantly decreased cardiac ALT activity, and elevated serum activity of the enzyme (*$p < 0.05$ vs control).

Fig. 4: Effect of pharmaceutical plant effluent on serum uric acid and creatinine in mice. There was an increase in serum uric acid and creatinine levels at concentrations 5 %, 10 %, 20 % and 40 % (*p < 0.05 vs control).

Table 1:
Effect of pharmaceutical effluent on electrolytes

Treatment Groups	POTASSIUM	SODIUM	CHLORIDE	BICARBONATE
Control	4.63±0.89$_a$	73.38±3.82$_a$	13.07±0.46$_a$	3.71±0.92$_a$
2.5 %	9.60±0.72$_b$	73.17±0.35$_a$	15.31±2.87$_{ab}$	17.94±9.32$_b$
5 %	6.12±1.18$_{ab}$	60.19±3.87$_b$	21.82±2.70$_{bc}$	17.66±3.50$_b$
10 %	6.45±1.50$_{ab}$	57.90±0.35$_b$	24.51±2.25$_c$	10.08±3.02$_{ab}$
20 %	6.86±1.05$_{ab}$	47.21±2.43$_c$	16.81±1.92$_{ab}$	11.72±7.24$_b$
40 %	4.30±0.81$_a$	58.35±2.01$_b$	15.44±1.67$_{ab}$	6.70±3.24$_a$

Values along the same column with different superscripts are significantly different (p < 0.05)

DISCUSSION

The results of this study show that, chronic oral exposure to pharmaceutical effluent can induce cardiac and renal dysfunctions by disrupting enzyme activities, raising serum uric acid and by causing electrolytes imbalance. Activities of ALP, AST and ALT in both tissues and serum are important biomarkers in determining organ integrity and function (Arise et al., 2012; Shahjahan et al., 2004). More so, correlation of ALP with C-reactive protein, inflammation, obesity, and atherosclerosis makes it a potential diagnostic marker or predictor of cardiovascular disease (Webber et al., 2010). During organ damage, these enzymes are leaked into the blood, causing increase in their serum levels, with subsequent decreased activities in the respective organs (Arise et al., 2012). Therefore, decrease in cardiac ALP, AST and ALT with concomitant elevated level of serum ALP indicates that, heart membrane was damaged, after chronic exposure to pharmaceutical effluent, and probably resulting in cardiac dysfunction. Decrease in cardiac AST and ALT activities was equally followed by a decrease in serum activity of the enzymes. This may mean that, the decrease in cardiac enzymes activities observed, was due to antagonistic effect of the effluent on AST and ALT production (Akanji et al., 1993). Previous studies have associated elevated serum ALP level with coronary artery disease (CAD), as it promotes vascular calcification through pyrophosphate pathway (Johnson et al., 2006; Schoppet and Shanahan, 2008). Furthermore, high level of serum ALP increases risk of mortality and unfavourable prognosis in coronary artery disease (Park et al., 2013; Wannamethee et al., 2013) as well as other cardiovascular diseases (CVDs) such as peripheral artery disease (Cheung et al., 2009), left ventricular hypertrophy (Nasri et al., 2004), secondary cardiac failure and diastolic dysfunction (Salgueira et al., 2005). Increase in serum ALP level observed in our study, therefore, suggests that, prolonged drinking of pharmaceutical effluent-contaminated water may promote the incidence of CVDs. Our finding is in consistence with our previous study (Abdulkareem et al., 2019) and the report of Karabulut et al. (2014).

Uric acid (UA) is a double bonded organic compound which, due to its antioxidant property, protects against oxidative stress (Sautin and Johnson, 2008). Elevated level of this compound however, may serve as an independent risk factor for coronary artery disease as it is frequently observed in patients with heart failure (Tian et al., 2012). Our results show that, in conjunction with elevated serum ALP level, oral exposure to pharmaceutical effluent at highest concentration (40 % v/v) elevated serum uric acid (SUA). This proposes further that, water contamination with pharmaceutical effluent may predispose an individual to cardiovascular diseases. Similarly, previous studies have reported increased SUA as an independent predictor of renal dysfunction in diseases such as rheumatoid arthritis and congestive heart failure (Daoussis et al., 2009; Tian et al., 2012). Therefore, the elevated SUA in our study may be a consequence of impaired renal function, which resulted to reduced excretion of UA. This thought is

supported further by concomitant increase in serum creatinine and electrolyte imbalance in our results (Figure 1a and Table 1).

Increase in serum creatinine in the exposed groups may be an indication of acute kidney injury, chronic kidney disease and renal dysfunction. Once creatinine is produced, it is immediately removed from the body by the kidney via urine. Since elimination of this molecule is solely by kidney, serum creatinine is widely used as a marker for renal function and readily employed in assessing acute kidney injury and chronic kidney disease (Winnett, et al., 2011; Baumgarten, 2011). Although, serum creatinine as a sole marker of kidney function has limitations; the observed electrolyte imbalance is a further confirmation of kidney dysfunction. The kidneys play a fundamental role in the regulation of body fluids and electrolytes; hence, impairment of kidney function results in derangement in electrolytes (Dhondup and Qian, 2017). Importantly, the observed hyperkalemia, hyperchloremia, hyponatraemia and elevated serum bicarbonate in the exposed animals have been earlier reported as predictors of chronic kidney disease and end-stage renal diseases (Dobre et al., 2015; Suetrong et al., 2016; Lim et al., 2016; Dhondup and Qian, 2017). Therefore, drinking water, contaminated with pharmaceutical effluent, may lead to development of chronic kidney disease and end-stage renal diseases. Interestingly, renal dysfunction has been noted to be a strong independent predictor of cardiovascular outcomes and mortality in the general population (Go et al., 2004), after myocardial infarction (Anavekar et al., 2004) and heart failure (Hillege et al., 2000). It can therefore be inferred from this study that, water contamination with pharmaceutical effluent may perhaps promote the pathogenesis of cardiovascular diseases via kidney function impairment.

The cardiorenal effects of pharmaceutical effluent observed in this study may be attributed to the combined effects of the chemical constituents. Chemicals such as Fe, Mn and NH_3 were found to be above permissible limit in our effluent sample. Meanwhile, iron overload has been reported to increase the fragility and density of renal lysosomes, thus, affecting their activities (Dimitriou et al., 2000). Since renal lysosomes play an important role in mediating kidney function of maintaining water-electrolyte homeostasis (Surendran et al., 2014), the disruption of serum electrolytes balance observed in this study may be due to renal iron accumulation. Similarly, cardiotoxicity and tissue damaging effect of Mn and NH_3, respectively, have been previously reported (Millera et al., 2006; McDaniel et al., 2016), hence, presence of these chemicals in the effluent probably resulted to impairment in cardiac enzymes activities.

The results of this study demonstrated that, drinking water, contaminated with pharmaceutical effluent, may disrupt enzyme activities, cause electrolytes imbalance and elevate serum uric acid. Therefore, such water may independently predispose individuals to cardiorenal syndrome. Further study, investigating the histology of concerned organs and mechanism(s) of cardiorenal toxicity of pharmaceutical effluent, should be carried out to exploit its roles in pathogenesis of kidney and cardiovascular diseases.

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

22-27 September, 2019, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

OMEGA - 3 FATTY ACIDS MITIGATE OXIDATIVE AND INFLAMMATORY EVENTS IN LEAD - TREATED RATS

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Lead remains a constant threat to our environment because of its non-biodegradable nature (Olayaki *et al*., 2018). Therefore, the study investigated the effects of omega-3 fatty acids (N-3) in lead-treated rats. Twenty male Wistar rats of five animals per group were used for this study. The control group received saline (0.1ml, *po*, daily) during the four weeks duration of the experiment, while group 2 received lead chloride ($Pbcl_2$) and saline during the first and last two weeks of the experiment respectively. Groups 3 and 4 were administered lead during the first two weeks; afterwards, they were treated with N-3 during the subsequent two weeks. $Pbcl_2$ was administered at 50mg/kg bw/day(*po*), while N-3 were administered at a low and high dose of 100 and 300mg/kg b.w./day(*po*) respectively. The results showed that there were significant elevations in the plasma levels of TNF-a, IL-6, CRP and MDA, and significant decreases in SOD, catalase and total antioxidant capacity (TAC) in the lead untreated group (Kim *et al*., 2007). Post-treatment with N-3 after lead exposure caused significant diminutions in TNF-a, IL-6, CRP, ROS, and MDA, and significant increases in catalase, SOD and TAC, relative to lead untreated group. The low dose of N-3 had more significant effects on TNF-a, IL-6, CRP, MDA and TAC, compared to the high dose. However, the latter demonstrated more significant effects on catalase, SOD and ROS. The study concluded that dietary supplementation with N-3, preferably at a low dose, mitigate the adverse effects of lead via antioxidative and anti-inflammatory mechanisms

MODULATORY ROLE OF SELENIUM YEAST ON OXIDATIVE STRESS BIOMARKERS OF CHOLESTEROL DIET INDUCED TYPE 2 DIABETES MELLITUS IN WISTAR RATS

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Selenium is an antioxidant that prevents oxygen radical from damaging cells from chronic diseases that can develop from cell injury and inflammation such as diabetes mellitus. The aim of the study is to investigate the possible protective effect of selenium yeast on oxidative stress biomarkers of cholesterol diet induced type-2 diabetes mellitus in rats. Twenty male wistar rats were divided in to four groups of five animals each: Group 1: (Negative control) received standard animal feed only, Group 2: received cholesterol diet (CD) only, Group 3: received CD and 0.1 mg/kg selenium yeast orally, Group 4: Received CD and 0.2 mg/kg selenium yeast orally for six weeks. At the end of the study period, the animals were sacrificed and the serum samples were collected and evaluated for estimation of blood glucose levels and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The results showed a significant ($P < 0.05$) decrease in blood glucose level in the groups co-administered CD and selenium yeast when compared to CD group only. Antioxidant enzymes status recorded significant ($P < 0.05$) decrease in SOD, CAT and GPx activities in CD and selenium yeast administered when compared to CD group only. In Conclusion, Selenium yeast administrations prevent free radical formations which are potent inducers of diabetes mellitus.

AGE-DEPENDENT CHANGES IN VASCULAR REACTIVITY IS INDUCED BY ARGINASE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Arginase activity reduces substrate (L-arginine) availability for nitric oxide formation while enhanced vascular arginase activity contributes to endothelial dysfunction in Dahl-S rats with salt-induced hypertension. There isa paucity of information on vascular arginase activity of aged normotensive and spontaneously hypertensive rats (SHR). This study tests the hypothesis that the age-dependent vascular endothelial dysfunction in thoracic aortafrom SHRs is mediated by Arginase. Acetylcholine (ACH) [10^{-9}–$3x10^{-5}$M] - induced concentration-dependent relaxation responses of thoracic aortic rings from young and old SHR and control ratswere evaluated, using wire myography, in the absence and following incubation for 30 minutes with L-Arginase (0.5U/ML). Endothelium-independent relaxation was also evaluated using sodium nitroprusside (SNP) [10^{-12} – $3x10^{-}$

$^{-5}$ M]. Vessels were pre-constricted with 10^{-6}M phenylephrine. Data are expressed as mean ± S.E.M. of 6 rats per group; statistical differences were calculated using Student's t-test and two-way ANOVA with repeated measures followed by Bonferroni post hoc test. Significance was set at $p<0.05$. ACH and SNP-induced relaxation responses were significantly decreased in aortic rings from old SHRs incubated with L-arginase compared with the rings without L-arginase ($p< 0.0001$) but were unchanged in the young SHRs. ACH and SNP relaxation responses were also unchanged in aortic rings from old and young control rats, in the absence or presence of L-Arginase [0.5U/ML]. Results suggest that arginase promotes vascular endothelial dysfunction in the old SHR as well as vascular smooth muscle dysfunction.

EFFECT OF HEXANE LEAF EXTRACT OF *LAUNAEA TARAXACIFOLIA*, RESVERATROL AND THEIR COMBINATION ON SERUM PROLACTIN AND OXYTOCIN LEVELS OF LACTATING FEMALE WISTAR RATS

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Lactogenesis, a neuro-endocrine event, is a complex neurophysiological process that involves interaction of a number of physical and emotional factors along with the action of multiple hormones, mainly prolactin. The aim of this study was to evaluate the comparative effects of *Launaea taraxacifolia* and resveratrol on serum prolactin (Prl) and oxytocin (Oxt) levels of lactating female Wistar rats. Twenty-five mature nulliparous female rats weighing 150-200 g were bred for the study. Following parturition, the number of pups per dam was adjusted to 5 and the dams were randomly allocated into five groups of 5 dams each. Dams in groups I, II, III, IV and V were administered distilled water (DW: 2 ml/kg) and metochlopromide (MET: 15 mg/kg), resveratrol (RES: 5: mg/kg), n-hexane leaf extract of *L. taraxacifolia* (LTF: 250 mg/kg) and their combination (CO; RES + LTF: 5+250 mg/kg) for 12 days (Cai *et al*., 2015). Serum was harvested and assayed with rat specific Prl and Oxt ELISA kits. The concentration of Prl was highest ($P < 0.05$) in LTF group when compared to other groups. Paradoxically, CO had lower ($P > 0.05$) concentration of Prl when compared to LTF and RES groups. However, Oxt concentration was highest ($P < 0.05$) in CO group when compared to other groups. The LTF group had higher ($P < 0.05$) Oxt concentration than MET and RES groups. In conclusion, *L. taraxacifolia* stimulated hyper prolactinaemia, while its combination with resveratrol caused increased serum oxytocin levels. Therefore, it is recommended that LTF could be beneficial as a galactogogue, while its coadministration with RES could serve as a potent stimulator of the milk let down reflex.

EFFECTS OF SOYA BEAN SUPPLEMENTS ON BLOOD GLUCOSE LEVELS AND LIPID PROFILE OF ALLOXAN INDUCED DIABETES MELLITUS IN WISTAR RATS

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Uncontrolled diabetes leads to several complications that affect many organs of the body. The health- beneficial effects of dietary fibres and antioxidants derived from plant food sources including soya beans are being extensively studied. This study aimed to evaluate the effects of soya bean supplements on blood glucose levels and lipid profile of alloxan-induced diabetes mellitus in Wistar rats. Twenty albino Wistar rats of both sexes between 120-150 grams were used for this study. Diabetes mellitus was induced by a single intraperitoneal injection of alloxan monohydrate at a dose of 150mg/kg body weight. Rats having fasting blood glucose levels of 200mg/dL and above after the induction were used for the study. The diabetic rats were grouped into four groups of five rats each: Group 1 (negative control) received distilled water orally for two weeks; Group 2 (positive control) were administered 5mg/kg body weight of glibenclamide orally for two weeks; Groups 3 and 4 were fed with 25% and 50% soya bean supplements respectively for two weeks. The fasting blood glucose levels were determined at intervals of 0, 1, 3, 6, 9 and 12 days respectively using a digital glucometer based on the glucose oxidase method. The animals were anaesthetised at the time of sacrifice by being placed in a sealed inhalation jar containing chloroform-soaked cotton wool. Blood samples were taken from all the groups for the determination of lipid profile. Data obtained were analysed using analysis of variance (ANOVA). The fasting blood glucose levels and lipid profile were significantly reduced ($P \leq 0.05$) in the soya beans supplemented group as compared with the control group after the two weeks of supplementation. **S**oya bean supplementation was found to have blood-glucose lowering potential and anti-lipidaemic activity in Alloxan-induced diabetic Wistar rats.

MODULATORY ROLE OF TAURINE IN ALLOXAN-INDUCED OXIDATIVE STRESS, BODY WEIGHT AND BLOOD GLUCOSE CHANGES IN WISTAR RATS

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In this study, the preventive effect of taurine on body weight, blood glucose level, oxidative stress and lipid peroxidation in alloxan-induced diabetes mellitus in wistar rats was determined. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Blood glucose level was measured after 72 hours of induction and diabetes was considered in animals with blood glucose level greater than 200 mg/Dl with Accu-Check Active Glucometer. The rats were grouped into (5) groups of five animals each n = (5) Group 1: Normal control, Group 2: diabetes untreated, Group 3: diabetes treated with 100 mg/kg taurine, Group 4: diabetes treated with 200 mg/kg taurine, and Group 5: diabetes treated with glibenclamide (1 mg/ml). Blood glucose and body weight of the rats were measured on weekly basis for three consecutive weeks. At the end of the treatment, all animals were sacrificed and blood samples were collected for serum extraction and determination of oxidative stress biomarkers and lipid peroxidation. The result of the present study shows a significant ($P < 0.05$) decrease in body weight and blood glucose level in groups that received 100 and 200 mg/ kg of taurine compared with diabetes untreated group alone at 21 days of the experiment. For oxidative stress biomarkers superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), P values < 0.05 were considered significant. Lipid peroxidation activities also showed a significant ($P < 0.05$) decrease in serum malondialdehyde (MDA) concentrations in the treatment groups. In conclusion, this study revealed that taurine could be a preventive supplement against oxidative stress in alloxan-induced diabetes and could be a potent supplement to mitigate against its occurrence.

EFFECT OF SELENIUM YEAST SUPPLEMENTATION ON SOME OXIDATIVE STRESS BIOMAKERS IN STZ-INDUCED DIABETIC NEUROPATHY IN WISTAR RATS

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Diabetic neuropathy is a long-term complication of diabetes, which affects half of the diabetic population. There is no prophylactic therapy against painful diabetic neuropathy. Current approaches are restricted to alleviating established pain by using drugs that are effective in other pain conditions and hoping to find one with enough efficacy and minimal side effect to be useful. The study investigated the effect of selenium yeast supplementation, an antioxidant and anti-inflammatory compound on some oxidative stress biomarkers in streptozotocin-induced diabetic neuropathy in Wistar rats. Thirty (30) adult Wistar rats with an average weight of 150 g were randomly divided into groups of five rats each (n=5). Diabetic neuropathy was induced by single intraperitoneal injection of 60 mg/kg streptozotocin dissolved in 0.1ml citrate buffer (pH 4.5). Groups I, II and III received 1mg/kg distilled water and aspirin (300mg/kg) while groups IV and V received 0.2 mg/kg and 0.3 mg/kg selenium yeast respectively. Blood plasma glucose concentration was determined weekly for four weeks. At the end of the fourth week, rats were euthanized and blood samples were collected and used to estimate for Malondialdehyde, glutathione peroxidase and superoxide dismutase concentration. The result showed that Streptozotocin (STZ) significantly ($p < 0.05$) increased MDA concentration while SOD and GPx levels were significantly reduced. Selenium yeast supplementation significantly enhanced antioxidant enzyme activity ($p < 0.05$). The results demonstrated that selenium yeast supplementation was beneficial in diabetic neuropathy via attenuation of oxidative stress. This suggests that Selenium yeast (0.2mg/kg) has potentials for the prevention of diabetic-induced neuropathy.

INVESTIGATION OF EFFECTS OF HYDROMETHANOLIC LEAF EXTRACT OF *CNIDOSCOLUS ACONITIFOLIUS* ON SOME LIVER ENZYMES AND HISTOLOGY IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS.

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The potency of plants are largely due to their phytochemicals constituents. These phytochemicals often times affects biochemical activities in animals and humans, thus the reason for the attention due to possible adverse effects or benefits, especially on liver enzymes and histology. This study is aimed at investigating the effects of hydromethanolic leaf extracts of *Cnidoscolus aconitifolius* on liver enzyme and histology in streptozotocin induced diabetic wistar rats. Thirty wistar rats with an average weight of 230grams were randomly assigned into five groups of six animals each. Group 1, served as negative control (non-diabetic) and received normal chow and water *ad libitum*, group 2 served as positive control group and received 10mg/kg bw of glibenclamide, groups 3, 4 and 5 served experimental group and received 100mg/kg bw, 150mg/kg bw and 200mg/kg body weight induced of C.A orally for 28days after being induced with diabetes using streptozotocin. Phytochemical screening of the extract revealed the presence of highly abundant level of alkaloids and flavonoids with moderate levels of tannins, phlobotannins, saponins, terpenes cardiac glycoside and cynogenetic glycoside. Administration of the extract shows significant ($p< 0.05$) increase in AST, ALT, ALP concentrations and alteration of the hepatic cells in the experimental group. The biological active phytochemicals in the hydromethanolic leaf extract of C.A may disrupt the liver enzymes, thus inducing cell rupture in the hepatocellular architecture, and might be hepatotoxic.

EFFECT OF AQUEOUS CRUDE EXTRACT OF *SENNA OCCIDENTALIS* LEAVES ON ANGIOTENSIN CONVERTING ENZYME (ACE) ACTIVITY IN HIGH-FAT-DIET INDUCED OBESITY IN WISTAR RATS

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Hypertension is one of the most common diseases across the globe responsible for many deaths resulting from some of its serious complications. There is a constant quest for better and safer medications in order to improve patients' quality of life. *Senna occidentalis* is extensively used in folklore medicine to treat several illnesses with little validation of its therapeutic benefits. Thus, the scientific evaluation of the effect of aqueous extract of *Senna occidentalis* on angiotensin converting enzyme (ACE) in obese Wistar rats was carried out in the present study. Samples from kidney, lung and heart tissues from 30 animals were used for the study which lasted for 3 weeks. Experimental animals were grouped into six (n=5) as normal control, obese control, Captopril 1.07mg/kgbw/day, obese + 100 mg/kgbw/day extract and obese + 250 mg/kgbw/day extract groups. Obesity was induced by feeding the animals with high-fat diet for eight weeks after which treatment was embarked upon. From the result of the present study, ACE activity was found to increase significantly (P<0.05) in lung, kidney and heart in obese control rats when compared to those of normal control group. ACE activity in kidney, lung and heart of obese Wistar rats treated with aqueous crude extract of *Senna occidentalis* at doses of 100mg/kg bw/day and 250mg/kg bw/day was found to decrease significantly (P<0.05) at the dose of 250mg/kg bw/day when compared to obese untreated group. ACE activity of normal group treated with 250mg/kg bw/day was found to decrease but not significantly (P<0.05) when compared to both normal control and obese untreated groups. The observed effect in the present study could attributed to active principles present in the extract. Thus, aqueous extract of *Senna occidentalis* can be relevant in the management of hypertension.

EFFECT OF L-CITRULLINE ON HYPERLIPIDEMIC WISTAR RATS

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Hyperlipidemia is a common predicament in society due to change of lifestyle and food practice.The study was designed to evaluate the effect of l-citrulline on hyperlipidemia. Twenty five male Wistar rats were divided into five groups of 5 animals each. (n=5) and treated for 21 days every 48hr interval. Group I (Normal Control), Groups II (Hyperlipidemic control), Group III (P-407+ Atorvastatin), Group IV (P-407+ 400mg l-citrulline), Group V (P-407+ 800mg l-citrulline). At the end of the experiment, blood samples were collected and the serum separated for evaluation of lipid profiles. L-Citrulline decreases total cholesterol (796.84±42.962 mg/dl) vs (732.21±13.77 mg/dl, 721.63±9.293 mg/dl) but however, was not significant (P < 0.05). Triglyceride level was significantly (p<0.05) decreased in a dose dependent manner (319.60 ± 11.58) vs (147.60±15.52, 139.40± 15.13) However, was not significant (P < 0.05). High density lipoprotein was increased (274.75±20.4) vs (319.30±27.2, 320.80±7.94) but however, was not significant (P < 0.05). Low density lipoprotein cholesterol decreased significantly (p<0.05) in a dose dependent manner (333.53 ± 30.24) vs (234.73±31.12, 175.30±8.21). In conclusion, L-citrulline decreases lipid profile in hyperlipidemia induced male wistar rats after 21 days oral administration.

COMPARATIVE EVALUATION OF GALACTOPOIETIC EFFECTS OF HEXANE LEAF EXTRACT OF *LAUNAEA TARAXACIFOLIA* AND RESVERATROL IN LACTATING FEMALE WISTAR RATS

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The inadequacy of milk supply to meet the increasing human population coupled with a decreasing livestock population has necessitated the need for a potent galactogoue, particularly from plant origin. This study was aimed at evaluating the galactopoietic effects of n-hexane leaf extract of *L. taraxacifolia* (LTF), resveratrol and their combination in Wistar rats. Twenty-five mature nulliparous female Wistar rats were bred and following parturition, the number of pups per dam was adjusted to 5. The dams and pups were divided into five groups of 5 dams each. The dams in groups I, II, III, IV and V were treated by gavage daily at 7:00 pm with distilled water (DW, 2 ml/kg), metochlopramide (MET, 15 mg/kg), resveratrol (RES, 5 mg/kg), LTF (250 mg/kg) and the combination of RES and LTF (CO, 5 + 250 mg/kg), respectively. The administration commenced on day 2 through to day 14 of lactation. Pups were weighed three times daily at 13:00 (W_1), 17:00 (W_2) and 18:00 (W_3) hours, respectively. The W_1, $W_3 - W_2$ and $(W_1 - W_0)/W_0$ denote daily weight of pups, milk yield and percentage growth rate, respectively. The results showed that milk yield was non significantly (P > 0.05) higher in CO and LTF than the yield obtained in RES, MET and DW groups. The daily weight gain of pups was significantly greater (P < 0.05) in LTF, RES and CO groups when compared to DW group. Although the LTF group had higher daily weight gain than the RES and CO groups, it was not significant (P > 0.05). However, percentage growth rate was significantly lower in MET and LTF groups compared to other groups; while RES had significantly higher percentage growth rate than CO. In conclusion, *Launaea taraxacifolia* and resveratrol exhibited galactopoietic potentials individually, and synergistically when co-administered.

IMPAIRMENT OF CARDIOVASCULAR FUNCTION INDICES IN MALE WISTAR RATS INDUCED BY ALUMINIUM-TAINTED WATER: ATHEROGENIC INDICES AND PREDICTOR RATIO ASSESSMENT

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Cardiovascular disease (CVD) epidemic is an ever-growing health problem and remains the leading cause of death in all regions of the world. Currently, there is growing evidence that exposure to metal pollutant is a risk factor that disturbs lipid metabolism with increase risk for CVD. The present work aimed to evaluate the toxicity of aluminium chloride - tainted drinking water ($AlCl_3$) on lipid profile in male wistar rats in assessing cardiovascular risk by using atherogenic indices and prediction ratio. Fifty (50) male Wistar rats were randomly assigned to five groups of 10 rats each. Group A was given normal drinking water whilst $AlCl_3$ treated groups B, C, D and E received 200, 400, 600 and 800mg/kg of $AlCl_3$ respectively via orogastric route once daily for 28 days. Thereafter, blood samples were collected for lipid profile analysis. Atherogenic indices like Castelli's Risk Index (CRI), Atherogenic Index of Plasma (AIP), and Atherogenic Coefficient (AC), lipid ratios and predictor ratios were determined. With values significantly different at $P \leq 0.05$, the estimated atherogenic indices- CR1-11, AC and CRI-1, ratios TG/HDL-c, TC-HDL-c/HDL-c, TC/HDL-c and low HDL-c/LDL-c, dose-dependently rose approximately and significantly differently determined cardiovascular risk in rats by $AlCl_3$. Predictor ratio however, revealed that AIP did not significantly impact the risk of cardiovascular disease in rats. In conclusion, exposure to $AlCl_3$ elicited concurrently dose-response proliferation of both dyslipidaemia and atherogenic indices differentially with resultant deleterious effect in cardiovascular cells and tissues in rats. AIP may not be an independent factor in $AlCl_3$ impacting the risk of CVD in rats. These might be the various possible mechanisms of aluminium toxicity in male rat cardiovascular risk.

CLOTTING TIME AND CALCIUM HOMEOSTASIS AMONGST POSTPARTUM HEMORRHAGE PATIENTS ATTENDING MURTALA MUHAMMED SPECIALIST HOSPITAL, KANO

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According to the World Health Organization, 15 women dies every hour as a result of obstetrics hemorrhage, with large proportion of these deaths occurring in developing countries. Several factors have been outlined as possible causes of postpartum hemorrhage (PPH) among which includes uterine atony. Serum calcium has been known to play a central role in vascular spasm, clotting mechanism and uterine tonicity, both of which affects uterine bleeding; hence disrupted calcium homeostasis may perhaps contribute to the incidence of PPH. Few local data is available on the relationship between PPH and clotting time, serum calcium and serum proteins. This study therefore assessed clotting time, serum calcium and phosphate levels as well as serum proteins among PPH patients and their normal delivery cohorts in Murtala Muhammad Specialist Hospital (MMSH), Kano, using a cross sectional comparative study design. Thirty (30) post partum hemorrhage patients matched with 30 cohorts of normal delivery were recruited after signing an informed consent. Blood samples were collected from the ante-cubital vein and transferred to a plain sample container for the estimation of serum analytes while clotting time was estimated using Dukes method. Sample analysis were done using specific kits obtained from Randox laboratories LTD, UK, all, according to the manufacturer's instructions. Data analysis was done using SPSS (V.20.0) and was summarized using mean±SD. An independent samples t-test was used to compare variables between the groups and statistical significance was set at 5% margin of error. The results indicated that PPH patients had significantly ($p=0.001$) higher clotting time, lower serum calcium, lower albumin and total serum protein than the controls. Serum phosphate was however not statistically different between the groups. In conclusion, the findings may point to an abnormality in calcium homeostasis of the PPH patients.

ASSESMENT OF MODULATORY ROLE OF CLOVE SUPPLEMENT ON LEAD INDUCED MEMORY IMPAIREMENT IN WISTAR RATS USING ELEVATED T AND Y MAZES.

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Cloves are dried flower buds of *syzygium aromaticum*, a tree of the myrtle family, which is evergreen tropical plant that flowers twice every year. It's an important medicinal plant due to it's wide range of pharmacological effects. Lead intoxication affects many systems of the body including the cardiovascular, renal, and reproductive systems. Its most detrimental effects occur in the nervous system, where it blocks N-Methyl-D-aspartate receptors. This study assessed the modulatory effect of clove supplement on lead induced memory impairment in Wistar rats. Twenty wistar rats (100-150g) were randomly divided into four groups (IIV) of five rats each. Group I served as control. Group II was treated with 0.1mg/kg of distilled water after pre-treatment with 10mg/kg of lead acetate. Grouped III was treated with clove supplement, 3% of clove supplement was mixed with the animal feed and 10mg/kg of lead acetate was administered. Group IV was also treated with clove supplement, where 6% was mixed with the animal feed and 10mg/kg of lead acetate was administered. Lead treatments were via oral gavage for 14 days. Neurobehavioural paradigms of Y and elevated plus mazes were employed to assess the spatial learning and memory. Findings revealed that, lead induced memory impairment was reduced at 6% and 3% respectively. It can be concluded from these results that lead induced memory impairment can be modulated using clove supplement.

EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF L-CITRULLINE IN MICE AND ITS ANTISPASMODIC EFFECT ON ISOLATED RABBIT JEJUNUM

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L-citrulline is a non-essential amino acid that plays a vital role in the metabolism and regulation of nitric oxide. It is majorly synthesized in the small intestine and considered safe for consumption. However, there is paucity of literature on its anti-diarrhoeal and antispasmodic effects. Hence, these study investigates the anti-diarrhoeal activity of L-citrulline in mice and its antispasmodic effect on isolated rabbit jejunum. Castor oil induced diarrhoea model was used for the antidiarrhoeal studies. Test groups received L-citrulline 300 and 600 mg/kg respectively, positive control group received Loperamide 5 mg/kg while negative control group received normal saline 2 ml/kg. All administrations were via oral route. The antispasmodic effect was evaluated using isolated tissue experiment. L-citrulline 300 and 600 mg/kg caused a reduction in the mean number of wet feaces when compared to the normal saline treated group. Diarrhoeal protections of 93.33% and 55.49% were observed at 300 and 600 mg/kg of L-citrulline, respectively. L-citrulline showed more anti-diarrheal effect at the lower dose of 300mg/kg and the result obtained was similar to that of Loperamide which showed higher percent protection. L-citrulline in the antispasmodic studies showed a concentration dependent decrease in strength of contractions of the rabbit jejunum which was similar to what was observed on administration of Adrenaline. When L-citrulline was interacted with Acetylcholine, there was a blockade of effect similar to that observed on interaction of the same concentration of Acetylcholine with Atropine. This indicates that L-citrulline might have inhibited the contractions of the rabbit jejunum via stimulating adrenoceptors and/or blocking cholinergic receptors. Therefore, from the results obtained in this study it may be suggested that L-citrulline possesses some antidiarrhoeal potentials.

SOME HEMATOLOGICAL INDICES AND LIVER FUNCTION PARAMETERS AMONG WORKERS EXPOSED TO CHRONIC INHALATION OF LIQUEFIED PETROLEUM GAS (COOKING GAS) IN CALABAR, NIGERIA

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Gas workers in Calabar, Cross River State are chronically exposed to Liquefied Petroleum Gas (LPG) or cooking gas. This gases being lipophilic in nature, after inhalation, are absorbed into the blood, and enters into the tissues of their body. This study investigated some hematologic and liver function parameters among workers exposed to chronic inhalation of LPG in Calabar. A total of ninety (90) male subjects consisting of 45 control and 45 LPG Workers were used for this study. FBC, liver enzymes, plasma proteins and bilirubin concentration were assessed. Air quality studies were carried out and questionnaires were filled. The results showed a significant increase in RBC, PCV, Hb conc. MCV, MCHC and MCHC of the test group when compared to the control ($P< 0.001$). A significant increase in the total WBC, Lymphocyte and ESR of the test group was found when compared to the control group ($P<0.05$).A significant increase in total bilirubin, Conjugated bilirubin ALP, and globulin ratio of the test group was observed when compared to the control ($P<0.001$), while a significant decrease in total protein and albumin ratio was found in the test group compared to the control group ($P<0.001$). Other health related complaints such as Fatigue, itches, headache, rashes, abdominal pains, jaundice and dizziness were also more common in the LPG exposed workers than the controls. In conclusion, chronic inhalation of LPG without necessary precautions causes several changes on hematological indices and liver function parameters of gas workers and could lead to blood diseases and liver malfunction.

BIOSIGNATURE OF TOLUENE DIISOCYANATE-INDUCED ASTHMA IN FEMALE BALB/C MICE: THE ROLE OF β-CATENIN MEDIATED AIRWAY EPITHELIAL INTEGRITY

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Highly reactive low molecular weight compound, toluene diisocyanate (TDI), is a common inducer of work-related asthma especially in industrialised nations. Oxidative stress, airway dysfunction and inflammation have been related to chemical-induced occupational asthma. The role of adherence junction proteins in the maintenance of airway integrity was previously reported. This study evaluated in-depth pathophysiology of TDI-induced airway epithelial desquamation as it relates to T-helper cell endotype. Female Balb/c mice were dermally sensitised and intranasally exposed to TDI following a 21-day exposure regimen. After the final exposure, bronchoalveolar lavage fluid (BALF), lung samples and serum were collected. Analysis of BALF leukocytes showed a significant rise in both total and differential leukocyte counts, with predominant airway neutrophilia. Similarly, BALF reactive oxygen species were significantly increased by TDI exposure. Lung tissue inflammatory cells infiltrates, mucus production and collagen deposition were similarly higher among TDI-exposed animals. In addition to epithelial desquamation, aberrant distribution of E-cadherin and β-catenin complexes were observed upon TDI exposure. A significant increase in Th2 cytokines (IL-4, IL-5 and IL-13) coupled with rise in IFN-γ, IgE and IgG inferred Th1/Th2 asthma endotype. Phenotypically, airway resistance and dynamic compliance were significantly altered by TDI-exposure. Whereas, the expression of upstream and downstream oxidative and inflammatory

mediators such as Akt, p38, Nrf2 activation and HO-1 were similarly affected by TDI exposure. These findings have delineated molecular targets for the management of chemical-induced asthma.

EVALUATION OF AGE- AND SEX-RELATED EFFECTS OF RAMADAN FASTING ON BODY WEIGHT OF APPARENTLY HEALTHY PERSONS IN KANO METROPOLIS, NIGERIA

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Fasting in the Month of Ramadan is physiologically a robust non-genetic, as well as non-pharmacological observance that is defined as a complete abstention from eating any solid foods or liquids every day between the months of May and June every year, or as the case may be depending on Geographical location. The purpose of the study was to investigate age- and sex-related variability in weight and BMI before and after Ramadan Fasting. Ages, weights and heights of all subjects were taken and BMI calculated using the *Adolphe Quetelet's standard formula (Kg/m2).* Paired sampling and independent sample t-test were used to determine changes in ages and sexual dimorphism, respectively. There were significant increases in body weight and BMI in male participants after fasting observances. Based on age group, the increase in weight and BMI was only significant among male participants within middle-aged. Gender wise comparison of weight and BMI revealed significant sexual dimorphism in weight only after Ramadan fasting, with higher mean value in males compared to females. However, sexual dimorphism based on age group showed significant differences in BMI among the middle age group, before the Ramadan Fasting, with higher mean value in females. It was concluded that, Ramadan Fasting has positive effects on body weight and BMI in middle-aged males; sexual dimorphism in body weight and BMI affects only the middle-age group.

EVALUATION OF POSSIBLE ANTI-DEPRESSANT EFFECT OF ALPHA-LIPOIC ACID ON CHRONIC MILD STRESS MOUSE MODEL OF DEPRESSION

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World Health Organization (WHO) reported that depression is the 4th highest contributor to the global burden of disease and is predicted to be the first by 2030. Alpha-Lipoic Acid (ALA) is a potential antioxidant; synthesized in the mitochondria, it has the potential to increase insulin sensitivity as one of its wide range of benefits. Increase in insulin sensitivity is found to elevate serotonin level via increasing its precursor tryptophan. This research was aimed to evaluate effects of ALA on chronic mild stress model of depression. Fifteen mice of both sexes were used in this study. They were grouped into three groups of five mice each. Group 1 received 10 ml/kg Normal Saline (NS), Group 2 received ALA 200 mg/kg and Group 3 received Flouxetine 20 mg/kg. At the end of two weeks application of Chronic Mild Stressors (CMS), treatment was administered for another two weeks. Tail Suspension Test (TST) was conducted before and after application of CMS and treatments. Result of TST after treatment revealed a statistically significant difference between ALA and NS in immobility time (behavioral despair). In Open Field Test (OFT), no statistically significant difference was observed between NS and treatment groups in line crossing (locomotor activity). In Novel Object Recognition Test (NORT), no significant difference was observed in percentage preference between NS and ALA but there was statistically significant difference between Flouxetine and NS. In conclusion, ALA 200 mg/kg has shown a potential anti-depressant like effect in TST by decreasing the immobility time of mice subjected to CMS but has no effect on locomotor activity (line crossing) and cognitive function as seen in OFT and NORT respectively.

EFFECTS OF PHOSPHODIESTERASE 5 ENZYME INHIBITORS ON COGNITION IN MALE ADULT WISTAR RATS

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Neurodegenerative diseases are said to be associated with dementia and treatment has not been fully successful. Phosphodiesterases (PDEs) are class of enzymes that reverse the formation of second messenger cyclic AMP and cyclic GMP thereby terminating the physiological response of their first messengers. PDEs inhibitors such as sildenafil and tadalafil are hypothesized to prolong the physiological action of glutamate thereby believed to partake in long term potentiating (LTP). In this study we explore the possibility of using these drugs to enhance memory. Twenty four 24 male wistar rats (150g) were divided into four groups of six each (n=6). Group I, II, III and IV received 10 ml/kg of distilled water (Negative control), 5 mg/kg of sildenafil, 5 mg/kg of tadalafil and 20 mg/kg of piracetam (positive control) respectively. All administrations were done via oral gavage for two weeks. Y maze and NORT paradigms were used to assay cognitive functions. The result of our finding showed a statistically significant decrease on short term memory in the sildenafil and tadalafil treated rats groups when compared with the control groups. Using Nobel Object Recognition Task (NORT), sildenafil and tadalafil treated groups were found have significantly increased preference core indicating memory enhancement when compared with the negative control group. The piracetam treated group which is a

standard memory enhancer showed statistically significant increase in the preference score compare to all other groups. In conclusion the study illustrates that sildenafil and tadalafil posses potentials for memory enhancement in rats treated groups. The study also indicated that tadalafil has more potent memory enhancement capability on the rats treated group.

ELECTROLYTE AND OXIDATIVE STRESS PROFILE OF HEALTHY ADULT POPULATION IN ZARIA, NIGERIA AND THEIR RELATIONSHIP WITH EXPERIMENTAL PAIN RESPONSE

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Oxidative stress (OS) results from accumulation of reactive species, beyond the endogenous anti-oxidant capacity. OS is known to impair physiological functions, which can alter health and wellbeing. This reactive species are detoxified by endogenous anti-oxidant enzymes, which help to keep the system in a state of homeostasis. Electrolytes are known to serve crucial functions in the body that include regulation of water balance, maintaining pH, and transport of substances. Electrolyte imbalance can be a marker of many disorders. This study investigated the electrolyte and oxidative stress profile of a healthy adult population in Zaria, Nigeria and their relationship with experimental pain outcome. Participants were apparently healthy adult volunteers between the ages of 20 to 65 years, and drawn from the city of Zaria and its environs. Experimental pain was induced using pressure algometry. About 5 ml of blood was collected for determination of serum electrolytes, MDA, GSH and SOD. The results showed that serum concentrations of sodium, potassium and chloride as well as oxidative stress profile did not vary with sex, age and ethnicity among the study population. There was a significant negative correlation between pressure pain threshold and serum concentration of potassium ($r = 0.2330$, $p = 0.003$) and chloride ($r = 0.2126$, $p = 0.007$), while serum sodium correlated positively ($r = 0.3439$, $p = 0.000$). Serum MDA, SOD and GSH did not show statistically significant correlation with pressure pain threshold ($p > 0.05$). In conclusion, serum electrolytes correlate significantly with experimental pressure pain threshold among healthy adult population in Zaria, Nigeria.

LEVELS OF CYTOKINES IN RELATION TO MATERNAL GROUP B *STREPTOCOCCUS* COLONIZATION AT DELIVERY

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Infection with Group B *Streptococcus* (GBS) causes high morbidity and mortality among newborn babies. Maternal GBS colonization during pregnancy is a leading risk factor for neonatal GBS disease. Apart from the culture and the polymerase chain reaction (PCR) methods recommended by Centers for Disease Control and Prevention, studies have shown that cytokines produced by immune cells in response to the presence of bacteria may also be useful at detecting bacterial infections. We therefore investigated whether maternal serum interleukin (IL)-6, IL-8 and IL-10 are useful indicators to predict GBS colonization at delivery. Healthy HIV negative pregnant women, free of any medical condition and not receiving antibiotics, were recruited for the study (n = 136). Vaginal swabs and venous blood were collected at the time of delivery. Swabs were used to isolate GBS using two methods: culture method and quantitative PCR. A mother was deemed colonized by GBS if either the PCR or the culture were positive. Maternal serum was used for cytokine analysis using high sensitivity premixed magnetic Luminex performance assays (R&D Systems). Cytokines values were normalized using a logarithm transformation. Of the 136 Participants, 47 (35%) were colonized with GBS. At delivery, there was no statistically significant difference in logged concentrations of IL-6 (P=0.8), IL-8 (P=0.5) or IL-10 (P=0.9) according to colonization status. Our results show that maternal serum IL-6, IL-8 and IL-10 are not reliable markers to predict GBS colonization at delivery.

IN VITRO AND *IN VIVO* ANTIDIABETIC PROPERTY OF MORINGA OLIEFERA CYCLOTIDE FRACTION

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Plant cyclotides are chemically stable and beneficial for medicinal application if they possess such properties. *Moringa Oliefera* exhibits anti-diabetic activity; however whether its cyclotide fraction also exhibits anti-diabetic effects is unknown. The present study was thus designed to determine the *in vitro* and *in vivo* anti-diabetic effect of *Moringa Oliefera* cyclotides (MOC). MOC fraction was obtained by solvent extraction and peptide purification. Toxicity assessment was carried out using the brine

shrimps lethality test (BST). *In vitro* antidiabetic property of MOC was assessed by α-amylase inhibitory activity using the dinitrosalicylic acid method. *In vivo* antidiabetic property of MOC was assessed in a type 2 diabetic (T2D) guinea pig model. BST of MOC was non-toxic (LC_{50}; 37561.3 µg/ml). MOC exhibited maximum α-amylase inhibitory activity of 88.3 % at 400 µg/ml. T2D animals presented elevated fasting blood glucose (FBG) (134.3±13.7 vs 89±9.8 mg/dl), reduced glucose utilization, normal insulin levels but reduced hepatic insulin receptor substrate-1 (IRS-1) (3.4±0.7 vs 11.1±1.7 %) and skeletal muscle Glut 4 expression (21.8±4.4 vs 30.5±5.1 %) when compared with control. Treatment of T2D animals with glibenclimide and MOC (10 mg/kg) reduced FBG to 108±11.3 and 105±13.1 mg/dl respectively, increased glucose utilization; increased expressions of hepatic IRS-1 and skeletal muscle Glut 4 ($P< 0.05$) respectively. While MOC treatment did not significantly alter plasma insulin levels in diabetic animals, glibenclimide significantly increased plasma insulin levels (0.68±0.04 vs 0.25±0.03 ng/ml). In conclusion MOC is nontoxic. MOC possesses anti-diabetic activity, probably mediated via inhibition of α-amylase activity and improvement of insulin action.

PINEAL AND HYPOPHYSEAL RESPONSES TO SELENIUM TREATMENTS IN LIGHT DEPRIVED ADULT FEMALE WISTAR RATS

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Dark is a generally known potent stimulus for melatonin secretion. Studies have also reported that pineal and hypophyseal endocrine secretions are influenced by nutritional factors. The study is designed to investigate the combined effect of selenium supplementations and light deprivation on pineal and hypophyseal secretions in female wistar rats. 36 female cyclical wistar rats were divided into vehicle, high selenium (HS), low selenium (LS), light deprived (LD), LD+HS and LD+LS. Rats were orally administered 150µg/kg and 100µg/kg of sodium selenite for two weeks. While light deprived rats were maintained under 6hr light/18hr dark cycle, other rats were under natural 12hr light/12hr dark cycle. The result showed that light deprivation led to significant decrease ($P<0.05$) in follicle stimulating hormone (FSH) secretion and a significant increase ($P<0.05$) in plasma melatonin. Selenium supplementations at both doses also improved LH secretion but had no effect on plasma prolactin. At high dose of selenium supplementation, there was an increase in melatonin secretion. In light deprived rats, selenium supplementations caused a significant decrease ($P<0.05$) in melatonin secretion at both doses but there was no significant change in plasma levels of FSH, LH and prolactin. The findings indicate differential responses of pineal and hypophyseal glands to light deprivation and selenium treatments.

AMELIORATIVE POTENTIAL OF *ALLIUM CEPA* ON P53 AND BCL2 EXPRESSION, DNA FRAGMENTATION IN LIVER OF CADMIUM SULPHATE TREATED RATS.

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Cadmium (Cd), one of the highly toxic metals to both humans and animals has been known to be cytotoxic and responsible for inducing free radical-dependent DNA damage in cells. *Allium cepa* is a known antioxidant with ameliorative potentials on cadmium-induced organ toxicity. Therefore this study was carried out to evaluate the effects of *Allium cepa* extract on DNA fragmentation, p53 and Bcl2 expression in liver of cadmium sulphate treated rats. Twenty adult male wistar rats weighing 160-180g were used for this study. They were divided into four groups, group one served as control, group two (Cd group) were treated with 15mg/kg $CdSO_4$, Group three (Cd + AcE group) were treated simultaneously with 15mg/kg $CdSO_4$ and *Allium cepa Extract* (1mL/100g BW) while the fourth group (AcE group) received *Allium cepa* extract (1mL/100g BW) alone. All the treatments were given orally for 28 days. Liver Superoxide Dismutase (SOD), Catalase (CAT) activities and % DNA fragmentation were examined spectrophotometrically while immunohistochemical expression of Tumor Suppressor protein (p53) and cytoplasmic Bcl2 were also studied. Exposure to cadmium significantly decreased SOD and CAT activities. It significantly increased % DNA fragmentation and Bcl2 expression and inhibited p53 expression. Decreased SOD and CAT activities, increased DNA fragmentation and Bcl2 expression together with inhibition of p53 expression by cadmium were all ameliorated with *Allium cepa* treatments. In conclusion, *Allium cepa* inhibits liver DNA damage by increasing antioxidant activities and inhibiting Bcl2 expression while promoting p53 expression.

AMELIORATIVE EFFECTS OF MANGANESE GLYCINATE AGAINST INCREASED LIVER ENZYMES IN RATS EXPOSED TO WATER IMMERSION RESTRAINT STRESS

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Stress response is crucial, as it enhances the organism's chances for survival. It may become overwhelmed by severe stress, resulting in organ injury, manifesting as diseases like gastric ulcers. Water-immersion restraint stress (WIRS) is known to cause increased levels of various serum enzymes in rats. The present study examined the effects of manganese glycinate on serum levels of the liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in rats exposed to WIRS. Fifteen (15) male Wistar rats (150-170 g) were divided into 3 groups of 5 rats each: (I) passive control; (II) active control; and (III) pre-treated with manganese glycinate and subjected to WIRS for 3.5 hours. At the end of the experiment, blood samples were collected through decapitation for haematological and biochemical analyses. The result showed that acute WIRS significantly ($p<0.05$)

increased plasma ALT, AST and ALP activities in the active control group, when compared with the passive control group. Pre-treatment of rats with manganese glycinate showed significant amelioration of these changes induced by WIRS. It was concluded that manganese glycinate pre-treatment has possible anti-oxidant and protective effects against liver damage as may be caused by WIRS in rats.

ANTIDEPRESSANT STUDIES OF METHANOL LEAF EXTRACT OF *Ziziphuss Mauritiana* IN ALBINO SWISS MICE

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The leaves of *Ziziphus mauritiana* have been reported to be used in the management of depressive illnesses in traditional medicine. The antidepressant activity of the methanol leaf extract of the plant was evaluated using tail suspension test and forced swim test at the doses of 25 mg/kg, 50 mg/kg, 100 mg/kg, and 200 mg/kg, the effect of the extract on recognition memory, motor coordination, and exploration behaviour was evaluated using novel object recognition test, beam walking assay test and open field test respectively. Data was and represented as± SEM and analyzed using one-way analysis of variance (ANOVA), followed by the Bonferroni *post hoc* test where P< 0.05 was considered significant. The result showed a statistical significant decrease in duration of immobility $p < 0.001$ on TST, when compared with the control. Imipramine (20 mg/kg) was used as standard antidepressant drug. It may be concluded that the methanol leaf extract of *Ziziphus mauritiana* possesses antidepressant property.

INFLUENCE OF AGE, SEX AND EDUCATIONAL STATUS ON SELF-MEDICATION AMONG FARMERS AND ITS ASSOCIATION WITH PEPTIC ULCER DISEASE

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The trend towards self-medication is at an increasingly alarming rate in our communities and it is becoming easier to procure more drugs over-the-counter (OTC) now than ever before. Peptic ulcer disease (PUD) incidence due to *Helicobacter pylori* infection is decreasing, however most cases now can be attributed to other secondary factors such as ingestion of Non-Steroidal Anti-inflammatory Drugs (NSAIDs). The readily availability of NSAIDS encourages the farmers to self-medicate. This study aims to assess the influence of age, sex and educational status on self-medication of NSAIDs among farmers in Shika District of Sabon Gari Local Government Area of Kaduna State. This was a descriptive cross-sectional study. A total of 220 questionnaires were administered. Study participants were sampled using a systematic random sampling technique and an interviewer administered structured questionnaire was used to collect data which was entered and analyzed using SPSS v17. About 91.8% of the farmers were found to self-medicate. Farmers ≤ 40 years were found to have about five times higher odds to self-medicate than farmers > 40 years of age (cOR=5.04; 95% CI 1.87- 13.58). Sex and educational status were not found to be statistically associated with self-medication. There is an alarmingly high prevalence of self-medication among young farmers. Younger farmers may have high risk of developing NSAID-induced peptic ulcer.

ASSESSMENT OF MILK YIELD AND SOME LACTOGENIC HORMONES IN FEMALE LACTATING WISTAR RATS FOLLOWING FERMENTED SOYA BEAN AND ASCORBIC ACID SUPPLEMENTATION

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Milk is essential for optimal feeding of infants and has a direct impact on growth, development, and health in neonatal period. This study was designed to assess milk yield following Soya bean and Ascorbic acid supplementation. At parturition, the animals were randomly divided into seven groups of five rats each (n=5) and treated as follows: Group I: (Normal control) was given normal feed and normal saline, orally (1 ml/kg bw), Group II: metoclopramide (5 mg/kg bw) ,Group III: 100 mg/kg bw of Vitamin C, Groups IV, V and VI were given soya bean supplement thus; 10%, 20% and 40%, respectively. Group VII was co-administered with 20% soya bean supplement and Vitamin C (100 mg/kg bw). Treatment was done for the period of ten (10) days at 06:00 hours daily and the animals were euthanized at the end of the experiment. Serum levels of prolactin, oxytocin and milk yield 18 and 23 hours after gavage were evaluated. The result on serum prolactin didn't show any statistical significant increase although there was increase in all the supplements treated groups compared to control except in the group administered 20% of Soya bean supplement. Serum prolactin level was highest in the Soya bean and Vitamin C co-administered group compared to control. Serum oxytocin level was statistically significant ($P< 0.05$) in the metoclopramide treated group (404.20±19.40 vs 342.80±20.30) compared to control. In this Soya bean supplement has been shown to increase serum prolactin and oxytocin level, resulting in a concomitant increase in milk yield

EVALUATION OF SOME LACTOGENIC HORMONES, MILK YIELD AND OXIDATIVE STRESS BIOMARKERS IN EXCLUSIVELY AND NON-EXCLUSIVELY BREASTFEEDING MOTHERS IN ZARIA

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The aim of the study was to evaluate some lactogenic hormones, milk yield and oxidative stress biomarkers in exclusively and non-exclusively breastfeeding mothers in Zaria. The population for this study consist of breastfeeding mothers attending postnatal care in Hayin Dogo primary health care centre Samaru - Zaria, aged 20 to 50yrs who practice different breastfeeding methods. A sample size of 60 was considered for the study. Five millilitre of venous blood was collected by vein puncture with sterile needle and the sera was used for hormonal and antioxidant assay. The result show a significant increase ($P< 0.05$) in milk index of the non-exclusive group when compared to the exclusive group; 0.11±0.012 (kg/child) vs 0.081±0.018 kg/child respectively. A significant increase ($P< 0.05$) in the serum prolactin level of the exclusive breastfeeding mothers was observed when compared to the non-exclusive breastfeeding mother 73.10±13.90ng/ml vs 45.67±14.33ng/ml respectively. Although there was an increase in the serum progesterone level of the non-exclusive breastfeeding mothers when compared to the exclusive breastfeeding mothers; 8.21±2.37ng/ml vs. 6.36±2.56ng/ml respectively, it was however not statistically significant. There was a non-significant increase ($P> 0.05$) in the serum estrogen level of the non-exclusive breastfeeding mothers (73.30±10.09pg/ml) when compared to the exclusive breastfeeding mothers (64.80±13.90pg/ml). Also, there were no statistical-significant increase ($P> 0.05$) in the serum MDA, SOD and GPx level of the exclusive breastfeeding mothers when compared to the non-exclusive breastfeeding mothers. It is therefore suggested that exclusively breastfeeding mothers should be well motivated and prepared to manage the stress associated with breastfeeding through up regulation of their antioxidant defence system.

COMPARATIVE STUDY OF THE ANTI-DIABETIC EFFECTS OF METHANOLIC EXTRACTS OF C*URCUMA LONGA* RHIZOMES AND S*PONDIAS MOMBIN* LEAVES IN ALLOXAN INDUCED DIABETES IN MALE WISTAR RATS

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Approximately eighty percent of deaths associated with diabetes mellitus the world over; occur in low and middle-income countries. Due to poverty, there is the tendency for patients to rely on orthodox medications with attendant adverse effects. Medicinal plants like *Curcuma longa* and *Spondias mombin* have been separately evaluated in ameliorating diabetic conditions. Therefore, this study attempts a comparative assessment of the anti-diabetic effects of methanolic extracts of C*urcuma longa* rhizomes and S*pondias mombin* leaves in alloxan-induced diabetic male wistar rats. Ninety rats were divided into nine groups of 10 animals each. Diabetes was induced using 200mg/kg/b.w of alloxan administered intraperitonially. The groups were treated as follows: 1: Non-diabetic control; 2: Untreated diabetic; 3: 200mg/kg/b.w *Spondias mombin;* 4: 400mg/kg/b.w *Spondias mombin;* 5: 500mg/kg/b.w C*urcuma longa*; 6: 1000mg/kg/b.w C*urcuma longa*; 7: 200mg/kg/b.w S*pondias mombin* + 500mg/kg/b.w Curcuma longa; 8: 400mg/kg/b.w S*pondias mombin* + 1000mg/kg/b.w C*urcuma longa*; 9: 0.6mg/kg/b.w. Glibenclamide. On day 43, blood was collected by cardiac puncture for determination of blood sugar and glycosylated haemoglobin concentration. A significant ($p< 0.05$) dose dependent decrease in the blood sugar and glycosylated haemoglobin levels were shown in all the treated groups compared to group 2. Furthermore, groups 7 and 8 showed a significant reduction ($p<0.05$) in blood sugar and glycosylated haemoglobin concentration compared to groups 3 to 6. S*pondias mombin* apparently showed better anti-diabetic effects compared to C*urcuma longa*. However, our result shows that combined treatment with S*pondias mombin* and C*urcuma longa* may potentiate the anti-diabetic effects seen in our experimental studies.

ACUTE AND SUB-ACUTE TOXICITY STUDIES ON METHANOLIC EXTRACT OF *Combretum dolichopetalum* LEAVES

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Combretum dolichopetalum leaves are used in African ethnomedicine as a blood booster, relief of menstrual pain, enhancement of labour, etc. However, to the best of our knowledge, systematic study regarding its toxicity profile has not been reported. The present study sought to carry out acute and sub-acute toxicity studies on *C. dolichopetalum* leaves. The studies were carried out on experimental mice and rats respectively using standard techniques. Results The LD50 of the extract was obtained as more than 5000 mg/kg body weight. Administration of graded doses (100, 200, 400 and 800 mg/kg) of the extract for 21 days resulted in increases in body weights, while blood cells (WBC), Neutrophils, red blood cells (RBC), packed cell volume (PCV), haemoglobin (HGB), mean corpuscular volume (MCV) and mean cell haemoglobin (MCH) of the rats; but did not affect ($P>0.05$) their monocytes, mean cell haemoglobin concentration (MCHC), platelet (PLT) levels. All doses of the extract did not affect ($P>0.05$) the sodium, potassium, chloride, bicarbonate, urea, creatinine, total and conjugated bilirubin, alanine and aspartate amino transaminase, aspartate amino transaminase, alkaline phosphatase activities; relative liver and kidney weights of the rats, a finding that was corroborated by histology of the liver and the kidney. The study provided a scientific rationale for the use of *Combretum dolichopetalum* leaves in Nigerian ethnomedicine as a blood booster. The study also revealed the safety in the usage of *C. dolichopetalum* leaves in Nigerian ethnomedicine

ADMINISTRATION OF FLAVONOIDS FROM *Hibiscus sabdariffa* TO SUCROSE-CONSUMING LACTATING RATS MAY PROGRAM INCREASED WEIGHT GAIN IN OFFSPRING LATER IN LIFE

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Incidence of metabolic dysfunction due to consumption of sugar sweetened beverage is on the increase (Miranda *et al*.,2005) and it is well established that its high intake by lactating dams is associated with development of metabolic dysfunction in offspring (Malik *et al*.,2010). *Hibiscus sabdariffa* (*HS)* with its phytochemicals, has shown prospects in prevention and treatment of obesity and metabolic dysfunction through unclear mechanisms (Alarcon-Aguilar *et al*.,2007). This study investigated the effect of administration of flavonoids from *HS* to sucrose consuming lactating rats on the offspring food intake and postnatal growth. 32 female Wistar rats were used for this study. They were divided into sucrose and non-sucrose groups and 8 sub-groups with 4 rats in each subgroup. Non-Sucrose groups were administered 10mg/kg, 20mg/kg and 50mg/kg of flavonoids from *HS*, the sucrose group were administered 30% sucrose with 10mg/kg, 20mg/kg and 50mg/kg of flavonoids from *HS* orally. Extract administration commenced on day 1 of lactation and ended on postnatal day 21. Blood samples were withdrawn from the offspring on postnatal day 42 for determination of leptin level. Results showed that administration of flavonoids from *HS* to lactating dams decreased preweaning and increased postweaning body weights and BMI, increased postweaning food intake of the offspring and decreased leptin concentration in the non-sucrose group whereas it increased leptin concentration in the sucrose group. It is concluded that maternal consumption of flavonoids from *HS* during lactation, with or without sucrose, may predispose the offspring to increased weight gain later in life through increased food intake.

EFFECT OF CONSUMPTION OF AQUEOUS LEAF EXTRACT OF *GONGRONEMA LATIFOLIUM* BY LACTATING WISTAR RATS ON SUCROSE-INDUCED PROGRAMMING OF METABOLIC DYSFUNCTION IN THE OFFSPRING

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Consumption of excess sugar has been implicated in the development of a number of metabolic abnormalities and the potential of herbs such as *Gongronema latifolium (GL)* has been increasingly recognized in prevention and treatment of human diseases. This research investigated the effect of consumption of aqueous leaf extract of *GL* by lactating Wistar rats on sucrose-induced programming of metabolic dysfunction in young adult offspring. 32 adult female rats were used for this study. They were divided into two groups; Non-sucrose treated group and Sucrose treated groups which were further subdivided into eight subgroups of four rats each representing the different concentrations of the extract as follows: Non-sucrose treated subgroup were administered 100mg/kg, 200mg/kg and 400mg/kg while the Sucrose treated groups were administered 30% sucrose with 100mg/kg, 200mg/kg and 400mg/kg of *GL* extract.. The extract was administered orally and daily throughout lactation. At PND 42, blood was withdrawn from both male and female offspring for estimation of serum lipid profile and insulin. Results showed that administration of sucrose with the extracts caused a dose related significant decrease ($P<0.05$) in offspring body weight, BMI, food intake, blood glucose level, decreased the Total cholesterol, triglyceride, LDL-C, VLDL, increased HDL. It was then concluded that consumption of aqueous leaf extract of *GL* during lactation probably has an abating effect on sucrose induced programming of metabolic dysfunction.

Na^+-K^+ PUMP ACTIVITY DIFFERENTIALLY MODULATES REACTIVITY OF ISOLATED RABBIT CAROTID ARTERIES EXPOSED TO ERYTHROCYTE CONSTITUENTS

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The possible involvement of vasospastic mechanisms in vaso-occlusive crisis of sickle cell disease remain unclear. This study aimed to establish if Na^+-K^+ATPase enzyme activity modulates vascular reactivity in response to phenylephrine (PE) in isolated rabbit carotid arteries exposed to erythrocyte constituents. Two (2) mm rabbit carotid arterial ring preparations were placed in 20 ml organ baths containing physiological salt solution (PSS) bubbled with 95% O_2, 5% CO_2, at 37^{o}C and pH 7.4 and isometric contractions measured, under an initial load of 2g. Arterial rings were equilibrated for 60 minutes and exposed to 50 µl of each erythrocyte constituents at an adjusted haematocrit of 0.6. Rings were exposed to K^+-free PSS in the absence (control) or presence of RBC constituents (ghosts, erythrocytes and haemoglobin solution) from Hb SS subjects for 30 minutes and contracted with 10^{-7} M PE. Re-introduction of K^+ (5 mM) to the bath would cause relaxation due to increased Na-K pump activity and hyperpolarization of the membrane. All results are presented as means ± SEM. The percentage

relaxation response to 5 mM K^+ observed in control and in the presence of SS GHOST, SS HBS and SS RBC were 43.28 ± 5.7, 15.46 ± 6.3, 19.77 ± 9.2 and 25.82 ± 8.9 respectively. 5 mM K^+ relaxation was differentially attenuated ($p<0.05$) in the order: SS GHOST > SS HBS > SS RBC. The results suggest a possible impairment of Na^+-K^+ ATPase enzyme activity in SCD, enhanced phenylephrine contractions by exposure to SS GHOST and a possible impairment of endothelial function.

COMPARATIVE EVALUATION OF THE EFFECT TWO ANTI-MALARIA DRUGS (CHLOROQUINE AND ARTEQUIN) ON SOME SERUM BIOCHEMICAL PARAMETERS IN RATS

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Artemisinin combination therapies (ACT) have replaced the old drugs (like chloroquine) used as first line treatment for malaria. This current study aimed to investigate the comparative effects of chloroquine (an old drug) and artequin (an ACT drug) on serum biochemical indices in rats. Thirty-six (36) Wistar rats were randomly assigned into 2 batches. Each batch had 3 groups of 6 rats each. Group 1 was control, groups 2 and 3 respectively received artequin (1.6mg/100g bwt) and chloroquine (0.875mg/100g bwt) orally and once daily. Administration lasted for 3 and 7 days for batches 1 and 2 respectively. The biochemical analysis of the serum was carried out using standard methods. Results obtained on both days 3 and 7 showed that serum total protein and globulin concentrations in the artequin group was significantly lower ($p<0.05$) compared to control. The alkaline phosphatase concentration in the artequin group on day 7 was significantly ($p>0.05$) higher compared to control. In conclusion, administration of artequin and chloroquine at their recommended doses and duration is relatively safe. Prolonged administration of artequin could predispose to low serum proteins and globulin with accompanied elevations in ALP levels while chloroquine could increase AST level signifying hepatocellular damage.

EFFECT OF CIMETIDINE ON HEMATOLOGICAL INDICES OF WISTAR RATS: MODULATORY ROLE OF VITAMIN C

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Cimetidine is a drug used in treatment of dyspepsia which is a clinical condition with widespread distribution. The aim of this study was to evaluate the effect of chronic cimetidine treatment at therapeutic dose on hematological indices and the modulatory role of vitamin C on any change induced by cimetidine treatment. Forty adult male Wistar rats were divided into four groups (n = 10) and treated orally for 60 days with distilled water (control); cimetidine (30 mg kg^{-1}); cimetidine (30 mg kg^{-1}) + vitamin C (25 mg kg^{-1}) and cimetidine (30 mg kg^{-1}) + vitamin C (50 mg kg^{-1}). At the end of the study blood was collected by heart puncture following adequate anesthesia. Total white blood cell (WBC) count (5.99 ± 0.20 x $10^3/mm^3$) and total serum protein (6.44 ± 0.21 g/dl) of the cimetidine-treated group were lower than that of the control (7.95 ± 0.29 x $10^3/mm^3$ and 7.26 ± 0.18 g/dl, respectively), while the value for red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC), color index (C.I) and erythrocyte sedimentation rate (ESR) of the two groups were not significantly different. Treatment with vitamin C prevented the cimetidine-induced decrease in total WBC count and total serum protein. It was concluded that chronic cimetidine administration at therapeutic dose caused a significant decrease in WBC count and serum protein; and no significant effect on RBC count, PCV, Hb, MCV, MCH, MCHC, C.I and ESR; and vitamin C at 25 mg kg^{-1} was more effective than at 50 mg kg^{-1} in reversing the decrease in WBC count and serum protein caused by cimetidine treatment.

EVALUATION OF TOXICITY OF ALUMINIUM-TAINTED WATER IMPACT IN MALE WSTAR RATS: OXIDATIVE STRESS IN HEART AND KIDNEY

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Aluminium is a ubiquitous element; comprising about 8% of the earth's surface; and it has no known beneficial effect in humans, but can enhance adverse health effects. The adverse effect of aluminium on body organs is incompletely understood, necessitating the need to better understand the mechanistic-link in its induced adverse health outcomes.This study evaluated the oxidative stress and established the predictor ratio of the negative impact of aluminium chloride - tainted water ($AlCl_3$) assessed by alterations in pro-oxidant/antioxidant in the heart and kidney of male Wistar rats. Fifty male Wistar rats were randomly assigned to five groups of 10 rats each. Control group was given normal drinking water, while the $AlCl_3$ treated animals were administered 200, 400, 600 and 800mg/kg of $AlCl_3$ orally, once daily for 28 days. Heart and kidney specimens were collected for assessment of oxidative stress markers-malondialdehyde (MDA) and protein carbonyl (PCO) and glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities. The results showed that $AlCl_3$ in a dose-response-organ specificity induced significantly ($P\leq0.05$) higher oxidant/antioxidant enzymes' alterations in rats as reflected by marked increased MDA level with a concomitant decreased GPx, SOD and CAT activity.

Correlation coefficient indicated that all the oxidative stress markers were significantly different upon comparing in both $AlCl_3$ and control groups. In conclusion, our data indicated that dose-response-organ dependent raised oxidative stresses are the possible mechanistic-link in $AlCl_3$ induced male rat cardio-renal toxicity. Additionally, the established predictor ratio of the interplay in the interrelationship of the damaged tissue biomarkers can contribute to evaluation of pathophysiology risk of $AlCl_3$ in heart and kidney.

IN-VITRO NEGATIVE CHRONOTOPIC AND INOTROPIC EFFECTS OF AQUEOUS LEAF EXTRACT OF OCIMUM BASILICUM (SWEET BASIL) ON ISOLATED PERFUSED RABBIT HEART

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Numerous cardiovascular health benefits have been attributed to Ocimum basilicum. The aim of this study is to explore the effect of aqueous leaf extract of Ocimum basilicum on force and rate of contraction of the heart using an isolated perfused heart system. In this study different concentration (10mg/ml, 25mg/ml, 50mg/ml) of the aqueous leaf extract of Ocimum basilicum were used and the experiment was carried out using the isolated perfused rabbit heart model. Standard drugs such as Adrenaline, Propranolol, Acetylcholine and Atropine were also administered and their results were compared with those of the extract. The standard drugs were administered before the extract and at each administration the heart was allowed to recover or reach a basal contraction before the next administration. The extract exerts a negative chronotropic and negative inotropic effect on the isolated heart tissue in a concentration dependent fashion ($p < 0.005$). In conclusion aqueous leave extract of Ocimum basilicum possess negative inotropic and chronotropic properties.

MODULATORY ROLE OF CLOVE SUPPLEMENT ON CHRONIC RESTRAIN STRESS INDUCED ALTERATION IN PAIN PERCEPTION AND INFLAMMATION IN WISTAR RATS.

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The use of herbal medicine is wide spreading and growing, clove is being used for different medicinal purposes such as pain reliever for tooth aches, antiseptic, antispasmodic, antiviral and antibacterial. This study aims to determine the modulatory role of clove supplement on pain perception, inflammation and biomarkers of oxidative stress in Wistar rats exposed to chronic restrain stress. Forty male Wistar rats weighing 100-120g were used for the study. The animals were divided into four groups; positive control group (no stress), negative control group (exposed to restrain stress for four weeks without supplement), third group were given 3% clove supplement with 97% standard feed, fourth group were given 6% clove supplement with 94% standard feed. The third and the fourth groups were both exposed to restrain stress for four weeks (chronic stress). In the tail flick test, clove exhibited more pronounced anti nociceptive effect in the 6% clove supplement group, while a mild reduction in pain response latency at 3% clove supplement group was observed. The result of the anti-inflammatory study revealed that 3% and 6% clove supplement groups possessed anti-inflammatory activity with a maximum inhibitory effect at the end of four hours (4hrs). This study also showed an increase in the serum level of *SOD in the supplement groups, while a decrease in MDA level across the treatment groups was observed. The results obtained from this study confirmed the potentiality of clove as an anti-inflammatory and analgesic agent, as well as a potential anti-oxidant.

ACUTE EFFECTS OF 90-MINUTE FOOTBALL MATCH ON RED BLOOD INDICES IN SEDENTARY YOUNG MALES.

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Previous study, had demonstrated that standard ninety-minute football match did not have adverse effects on blood rheology in regularly trained male footballers. In this study, we aimed to investigate the acute effects of a single 90-minute football match on red blood cell indices in sedentary young males. This quasi- experimental study was carried out on 20 healthy males (20.00±0.48years old). The university institutional review board gave approval for all procedures in accordance with the Declaration of Helsinki. Fasting blood (3ml) was collected from antecubital vein before and after the 90 minutes football match. From the samples, pre- and post-match red blood cell indices were determined using standard methods. Data were analyzed by SPSS version 20 using t student and paired t-tests in order to compare group before and after exercise training. The 90 minutes football match decreased Red Blood Cell count and Packed Cell Volume significantly ($P \leq 0.05$). But it increased the Plasma Volume significantly ($P \leq 0.05$). Results of this study showed that an acute effect of a single 90-minute football match maintains red blood cells and pack cell volume are within physiological range in sedentary young men, thereby optimizing microcirculation to enhanced oxygen delivery to the working muscle.

EFFECTS OF ZINC AND FOLIC ACID ON SPERM MOTILITY AND TUMOUR NECROSIS FACTOR-ALPHA (TNF-A) LEVELS IN TESTICULAR ISCHAEMIC-REPERFUSION INJURY IN WISTAR RATS

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Ischaemic-Reperfusion Injury is a complex phenomenon that induces cell damage through a biphasic process-Ischaemia and reperfusion. The reperfusion phase of this process results in generation of reactive oxygen species and pro-inflammatory cytokines which affect sperm motility. This study attempts to investigate the ameliorative effect of zinc and folic acid on sperm motility and TNF-α levels following IRI. Thirty male Wistar rats were divided into six groups comprising of five animals (n=5). Group 1(control) were given 1 ml/kg normal saline for 21 days. Group 2 (sham) were given normal saline for 21 days followed by sham treatment. Group 3 (torsion/detorsion) were given normal saline for 21 days followed by torsion-detorsion. Rats in Group 4 (folic acid) were treated with (2 mg/kg)folic acid for 21 days followed by torsion-detorsion of the testis. Group 5 (zinc) were treated with (50mg/kg) zinc for 21 days followed by torsion-detorsion .Group 6 (Aspirin) were treated with (200mg/kg) aspirin for 21 days and induced with torsion-detorsion. At the end of 21 days, the epididymises were harvested and sperm motility investigated. Blood samples were collected, the sera harvested and used for TNF-α assay. The number of the non-motile cells decreased significantly ($p<0.05$) in the folic acid and zinc treated groups compared with the control. There was no significant difference in the TNF-α levels in the treated groups but a slight decrease was recorded in the zinc treated group. Results from this study suggest that oral treatment with folic and zinc could significantly reduce the adverse effect of IRI

ALLOXAN-INDUCED DIABETES CAUSES LIVER FUNCTIONS AND LIPID PROFILE CHANGES IN ALBINO WISTAR RATS: ROLE OF ETHNOLIC LEAF EXTRACT OF *GUIERA SENEGALENSIS*

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In most of African countries, a large number of diseases are treated administering plant infusions. Guierasenegalensis (Gs) has been used treats many diseases in Northern Nigeria, but its effects on liver function test, blood glucose level and lipid profile in diabetic rats has not been documented. Thirty five male albino rats with average weight of 200 g-250 g were used for study. They were divided into five groups of seven rats each: Group A (normal control). Group B (diabetic control) Groups C and D and E were induced with diabetes and treated daily with 100mg/kg, 150 mg/kg and 200 mg/Kg body weight of Gs leaf extract respectively for three weeks. At the end of this experimental procedure, rats were anaesthetized with diethyl-ether vapour. Blood samples were collected through cardiac puncture for measurement of serum metabolites. The result demonstrated that administration of ethnolic leaf extract of Gs in the induced diabetes rats has not shown any significant changes in the activities of ALP, AST and ALT when compared with diabetic control. However a significant increase was recorded in the serum total protein (TP) and albumin (ALB) when all the doses of Gs were compared with diabetic control. Administration of ethnolic leaf extract of Gs to alloxanized diabetic rats at the doses considered possesses hypoglycemic activities.

EFFECTS OF CALCITRIOL SUPPLEMENTATION ON RENAL, LIVER AND LIPID PEROXIDATION BIOMAKERS, IN FRUCTOSE-DRINKING ALBINO WISTAR RATS

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Type 2 diabetes mellitus is an important public health problem. The study was designed to examine the effects of oral calcitriol treatment on indices of renal and liver failure, antioxidant status and lipid peroxidation in fructose-drinking rats. Animals (130- 200 Kg) were randomised into four groups of five rats each and subjected to 125 µg/Kg body weight calcitriol treatment for three weeks after five weeks of fructose drinking. *Group I:* Control; normal rat feed + distilled water, *Group II:* normal rat feed + 125 µg/Kg body weight of Calcitriol, *Group III:* normal rat feed + 10% fructose solution, *Group IV:* normal rat feed + 10% fructose solution + 125 µg/Kg body weight of Calcitriol. All the parameters were determined using available commercial kits. Results showed that fructose-drinking rats exhibited significant increased in urea, creatinine, liver enzymes activities and lipid peroxidation index while the activities of Superoxide dismutase (SOD) and Catalase (CAT) were significantly reduced compared with the control. Calcitriol treatment significantly reduced renal failure indicators and activities of liver enzymes. However the activities of antioxidant enzymes were significantly increased. it was contrived that calcitriol treatment exhibited a reno-hepatic protection, enhanced the activities of the antioxidant enzymes and prevented lipid peroxidation in fructose-drinking rats.

THE EFFECT OF ADMINISTRATION OF CURCUMIN DURING SUCKLING ON THE DEVELOPMENTAL PROGRAMMING OF METABOLIC FUNCTION IN MALE AND FEMALE ADOLESCENT SPRAGUE DAWLEY RATS

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The current global epidemic of metabolic syndrome is linked to the increased consumption of fructose rich diets. Nutritional perturbations with natural polyphenols during the suckling period have demonstrated beneficial metabolic programming effects. Curcumin is a dietary spice that has anti-diabetic, antioxidant and anti-inflammatory properties. We aimed to determine whether administration of curcumin to rats during suckling would protect or predispose them to the adverse effects of a post weaning high fructose diet. Male and female Sprague Dawley pups (n=128) were allocated to four treatment groups and administered either a 0.5% dimethyl sulfoxide solution, curcumin (500mg.kg^{-1}), fructose (20%, w/v) or a combination of curcumin and fructose daily via oral gavage from postnatal days 6 to 21. All the rats were weaned unto normal rat chow and each of the initial groups was further subdivided into two subgroups; one group had plain tap water while the other had fructose (20%, w/v) as their drinking solution for six weeks. The rats were then fasted overnight and euthanised on postnatal day 63. Blood was collected via cardiac puncture and used for metabolic substrates and hormonal assays. There were no differences ($p>0.05$, ANOVA) in the fasting blood glucose, triglycerides, cholesterol, plasma concentrations of insulin, adiponectin and the homeostatic model of insulin resistance across the treatment groups. Administration of curcumin during suckling, a critical window of developmental plasticity in the rat, did not affect the metabolic response of adolescent rats to a post weaning high fructose diet.

EVALUATION OF THE ROLE OF ELLAGIC ACID ON MOTOR AND OXIDATIVE RESPONSES IN PENTYLENETETRAZOLE-KINDLED RATS

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Epilepsy is a neurologic disorder affecting more than 50 million people worldwide. Motor function impairment is among the disorders caused by epilepsy. Oxidative stress plays part in epileptic kindling. This study assessed the motor strength role of ellagic acid in pentyleneterazole (PTZ)-kindled rats. Thirty male Wistar rats (200 – 300g) with 6 groups of 5 rats each were used. Groups 1 – 5 received 35mg/kg PTZ, while group 6 received distilled water (*s.c*) on alternate days. One hour before PTZ administration, group 2, 3 and 4 received 15, 30 and 60 mg/kg (*p.o*) ellagic acid dissolved in 10% Dimethylsulphoxide (DMSO) respectively while group 5 received 30mg/kg phenobarbital (*i.p*) and were observed for seizure activity after PTZ injections. When kindling was achieved, motor strength test was conducted after which the rats were anaesthetized and their brain tissues used for assaying malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Results showed no significant ($P < 0.05$) improvement in motor strength in all the treated groups. A significant decrease ($P < 0.05$) was observed in MDA concentration in the treated groups when compared to control group 1. Also SOD increased significantly ($P < 0.05$) in all the groups when compared to control group 1. GPx was significantly increased ($P < 0.05$) in group 4 when compared to group 1 control. Findings from the study showed that epileptic seizure did not affect motor strength while oxidative stress was involved in kindling. Ellagic acid may be a potent antiepileptic drug with no associated side effects.

EVALUATION OF THE EFFECTS OF *CANNABIS SATIVA* L. EXTRACT ON MOTOR FUNCTION OF MALE WISTAR RATS

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The present study was aimed at investigating the effect of *Cannabis sativa* L. administration on motor coordination activity of adults Wistar rats. The work was limited to motor activity test of the cerebral cortex of adult male Wistar rats. Fifteen male adults Wistar rats of average weight 220g were divided into 3 groups with 5 animals per group. Animals in group 1(control) were given distilled water, while group 2 and 3 were administered with 250mg/kg and 500mg/kg b.wt respectively via oral route, daily for 21 days. Motor exploratory activity was assessed using Ladder rung walking test method. Motor assessment showed a significant increase ($P<0.05$) in the meantime taken to reach the home cage in groups 2, and 3 when compared to the control. It can therefore be concluded that *Cannabis sativa L.* administration result in motor exploratory impairment which could be due to degenerative changes in the cerebral cortex of adults Wistar rats.

THE HYPOTENSIVE EFFECT OF *HIBISCUS SABDARIFFA* TEA MAY OCCUR THROUGH THE INHIBITION OF THE DISCHARGE OF THE SYMPATHETIC NERVOUS SYSTEM

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This study tested the hypothesis that the hypotensive effect of *Hibiscus sabdariffa* tea (HST) may be due to its

attenuation of discharge of the sympathetic nervous system (SNS). Using a double-blinded placebo-controlled design, the hand grip exercise (HGE) was used to activate the SNS.[1] Following ethical approval, HGE was performed in healthy human subjects before and after the oral administration of 200mg/kg HST (n=20) or food colourant (n=20) which served as placebo. The basal blood pressure (BP) and pulse rate (PR) were obtained, then each subject held the hand-grip dynamometer forcefully at 30% maximal voluntary contraction (MVC) for 1-2 minutes until the onset of fatigue, and the BP and PR responses were measured. The mean arterial pressure (MAP) was taken as representative BP. Results were expressed as Mean±SEM and $P<0.05$ was considered significant. In the presence of HST, the HGE-induced changes (ΔMAP=8.7±1.3mmHg; ΔPR=8.4±1.0 beats/min) were significantly reduced compared to its absence (ΔMAP=15.0±1.8mmHg, ΔPR=14.5±1.5 beats/min; $P<0.0001$ respectively). However, in the presence of the food colourant, these changes (ΔMAP=11.2±0.6mmHg, ΔPR=11.9±0.8 beats/min) were significantly higher compared to its absence (ΔMAP=8.7±0.7mmHg, ΔPR= 9.7±0.7 beats/min; $P<0.0001$ respectively). These results suggest that HGE-induced activation of the SNS was attenuated by HST but aggravated by the food colourant. It is concluded that the hypotensive effect of HST may occur through the inhibition of SNS activation while the food colourant may further activate it.

EFFECT OF CIGARETTE SMOKING ON COGNITIVE FUNCTION AMONG YOUNG MALE UNDERGRADUATES IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

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About 2 billion people worldwide smoke tobacco product mostly in form of cigarette. Evidences on the effects of cigarette smoking on cognition have been very conflicting. While some studies have shown cigarette smoking to be protective against cognitive impairments other studies have shown otherwise. The aim of this study is to assess the relationship between cigarette smoking and cognitive impairment in young male undergraduate students of Ahmadu Bello University, Zaria, Nigeria. This is a cross-sectional study, comprising 62 smokers and 41 never-smokers as volunteers. Each volunteer was randomly selected and subjected to four cognitive battery tests which include the mini-mental state examination (MMSE), clock-drawing test (CDT), trail-making test (TMT) and verbal fluency test (VFT), with an inter-test period of about 15 minutes. Analysis was done using the SSPS v17. Most of the volunteers were within 18-27 years of age (50 [80.6%] of smokers and 35 [85.4%] of never-smokers). On individual cognitive battery test, varying degrees of cognitive impairments were found among both groups: MMSE (3.2% of smokers, none of never-smokers); VFT 1 (9.7% of smokers, 9.8% of never-smokers); VFT 2 (32.3% of smokers, 17.1% of never-smokers); CDT (38.7% of smokers, 14.6% of never-smokers); TMT A (71.0% of smokers, 75.6% of never-smokers) and TMT B (46.8% of smokers, 51.2% of never-smokers). Overall, the never-smokers appear to performed better than the smokers except in the cognitively-tasking TMT but only 16.1% of the smokers had cognitive impairment, as against 26.8% (11) of the never-smokers. Chi-square analysis revealed no association between smoking and cognitive impairment. Pearson's correlation also revealed weak correlations between smoking and cognitive impairment in all the tests. Therefore, light to moderate cigarette smoking confers some protective effect against cognitive impairment probably due to the cognitive-enhancing effect of nicotine contained in the cigarette smoke.

THE AWARENESS OF PHYSIOLOGY AS A SCIENTIFIC DISCIPLINE AND A PROFESSION AMONG SENIOR SECONDARY SCHOOL STUDENTS IN ABUJA, NIGERIA

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The recognition of Physiology as a profession and major discipline in medicine and scientific research in Nigeria is gaining momentum. This attention is however low especially among prospective university applicants. This study was conducted to determine the level of awareness of Physiology among secondary school students in the six local government areas of Abuja. The research was carried out in twelve randomly chosen secondary schools (private and public) in Abuja, Nigeria. One hundred and twenty (n=120) senior secondary school students (SSI-SSIII) were asked to fill in questionnaire, of which ninety-eight (98) responded. The first part of the survey included personal data and brief history. The second part contained 9 close-ended questions assessing students' knowledge about physiology as a discipline. The results were treated statistically using student's t-test. Apparently, data from the respondents shows that private and public schools are not familiar with the varied facets of Physiology as a discipline. Among the students in the private school, a mean of 2.18 for male and 2.29 female were obtained; while in public schools, a mean of 2.43 for male and 2.52 for female were obtained respectively. However, all the respondents were aware that there are professionals called Physiologist (private school: Mean $_{male}$ = 3.5; Mean $_{female}$ = 4.31 and public schools: Mean $_{male}$ = 3.0; Mean $_{female}$ = 4.31).The results of this study shows that most senior secondary school students are not very familiar with Physiology as a scientific discipline. However, their knowledge of Physiology as a profession was remarkable.

MODULATORY EFFECTS OF ACETONE EXTRACT OF Combretum micranthum BARK ON CYCLOOXYGENASE ENZYME

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Inflammation is an essential response provided by the immune system that ensures the survival during infection and tissue injury. Inflammatory responses are essential for the maintenance of normal tissue homeostasis. Combretum micrathum is used traditionally in Senegal and Mali for fatigue, liver ailments, headache, convalescence, diabetes, blood disease, weight loss, cancer, sleep disorders, most of this diseases are associated with inflammation. The aim of this study was to validate the numerous uses of Combretum micranthum plant. The acetone extract of Combretum micranthumwas screened for cyclooxygenase (COX) activity in a 96 well microtitre plate using COX screening assay kit. The result obtained demonstrated a weak inhibitory activity by the extract on both COX-1 and COX-2 with median inhibitory concentration values of 52.45±3.4µg/ml and 66.01±4.7µg/ml respectively. This shows that the extract had a weak anti-inflammatory property which may be attributed to the presence of some phytochemicals such as resins and glycosides in the extract. The median lethal concentration (LC50) was 41.32±0.92µg/ml which signifies low toxicity compared to the control drug doxorubicin with an LC50 value of 3.8±0.08µg/ml. In conclusion, the acetone extract of cambretum micranthum bark was found possess weak anti-inflammatory activity and low toxicity to vero cells.

LIVER ENZYMES AND SERUM ELECTROLYTES EVALUATION IN FEMALE LACTATING WISTAR RATS FOLLOWING FERMENTED SOYA BEAN AND ASCORBIC ACID SUPPLEMENTATION

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In mammals, lactation is the most energetically demanding period of a female's reproductive life. However, some of the substances used to enhance lactation have side effects. This study was designed to investigate the effect of fermented Soya bean and ascorbic acid supplements on some liver enzymes and histology. At parturition, the animals were randomly divided into seven (7) groups of five (5) rats each (n=5) and treated as follows: Group I: (Normal control) was given normal feed and distilled water, orally (1 ml/kg bw), Group II: metoclopramide (5 mg/kg bw), Group III: 100 mg/kg bw of Vitamin C, Groups IV, V and VI were given soya bean supplement thus; 10%, 20% and 40%, respectively. Group VII was co-administered with 20% soya bean supplement and Vitamin C (100 mg/kg bw). Treatment was done for the period of ten (10) days at 06:00 hours daily. The result on serum ALP level showed a significant increase in all the supplements treated groups; SB 10% (110.80 ± 1.63), SB 20% (127.60 ± 9.60), SB 40% (122.80 ± 2.60) and SB 20% + VIT C (129.40 ± 4.90) compared to the controls (86.60 ± 3.73) ($P< 0.05$). Although there was increase in the level of serum calcium, sodium, magnesium, potassium, chloride and bicarbonate in all the treated groups, it was however not significant compared to the control and metoclopramide treated group. The co-administration of the soya bean supplement and Vitamin C showed a significant increase in serum ALP, ALT and AST levels which could infer a detrimental potential of such combination to the hepatocytes.

EFFECTS OF HYDROMETHANOL EXTRACTS OF *Garcinia kola* ON SOME BIOCHEMICAL PARAMETERS OF MALE WISTAR RATS.

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Garcinia Kola is commonly used in traditional medicine for the treatment of diverse ailments including coronary artery diseases. Thus, this study aims to determine the effect of hydromethanol (1:4) extracts of the pulp and seed coat of *Garcinia kola* on serum lipid profile and its antioxidant properties. The two forms were separately dried and blended to powder. Forty male wistar rats (8 per group) were assigned into Five (5) groups. Groups were treated thus: Group one; control. Group two; 100mg/kg pulp extract. Group three; 200mg/kg pulp extract. Group four; 100mg/kg seed coat extract. Group five; 200mg/kg seed coat extract; for 30 and 60 days duration. On treatment conclusion, blood was collected for the determination of lipid profile and antioxidant properties. The higher dose of the pulp and seed coat extracts significantly ($P<0.05$) increased the catalase level and superoxide dismutase enzyme activity, whereas, both the higher and lower doses of the seed coat extract caused a reduction in malondialdehyde level. The serum total cholesterol was significantly elevated by the higher dose of the pulp extract while the seed coat extract caused significantly increased high density lipoprotein cholesterol level and a reduction in the low density lipoprotein level. The two extracts demonstrated marked antioxidant effects. The seed coat of *Garcinia kola* may possess the potential to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The seed coat extract may also be useful in preventing coronary artery disease and other atherosclerotic problems.

BLOOD GLUCOSE AND OXIDATIVE STRESS BIOMARKERS ASSESSMENT IN ALLOXAN INDUCED DIABETIC WISTAR RATS TREATED WITH QUERCETIN

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The study was designed to evaluate the effect of Quercetin on blood glucose level and oxidative stress biomarkers in Alloxan-induced diabetic wistar rats. The animals were divided into six groups of five each (n=5). Group I served as control and received distilled water, group II received dimethylsulphoxide 1% (DMSO), group III and IV received Quercetin at 50mg/kg and 100mg/kg respectively. Group V received vitamin C (100mg/kg) while group VI was given glibenclamide (1 mg/kg). Diabetes was induced by injection of alloxan 150 mg/kg intraperitoneally. All administrations were done via oral gavage for 21 days. Quercetin at a dose of 50mg/kg and 100mg/kg significantly (P<0.05) reduced fasting blood glucose level when compared to control (101.00 ± 7.27 vs 122.56 ± 8.02 mg/dL) vs(332.80mg/dL ± 36.53mg/dL). Serum catalase was significantly higher in the quercetin treated groups compared to the diabetic control. Serum level of SOD was significantly higher in the 100 mg/kg quercetin treated group relative to the diabetic control 2.44 ± 0.07 and 2.10 ± 0.05 vs 0.92 ±0.05. Serum malondialdehyde concentration was significantly lower (P<0.05) in the groups treated with qurcetin compared to the diabetic control 0.98 ± 0.01 vs 1.32 ± 0.06,. In conclusion oral administration of quercetin has been found to reduce blood glucose level, increase serum antioxidant level and significantly decrease serum malondialdehyde level in Alloxan-induced diabetic wistar rats.

THE EFFECT OF AQUEOUS AND N-HEXANE EXTRACTS OF *NIGELLA SATIVA* IN ALUMINIUM CHLORIDE –INDUCED HEMATOLOGICAL ALTERATION AND OXIDATIVE STRESS.

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Aluminium Chloride ($AlCl_3$) is a neurotoxic substance that has been known to induce hematological alteration and oxidative stress. The present study was designed to investigate the protective effect of *Nigella sativa* against $AlCl_3$ induce hematological alterations and oxidative stress in adult Wistar rats. Thirty adult Wistar rats divided into six groups of five animals each. Group 1 (control group) was given Tween 80%; Group 2 was given Aqueous *Nigella sativa* (ANS) extract at dose of 200mg/kg; Group 3 was given Hexane *Nigella sativa* (HNS) extract at dose of 50mg/kg ; Group 4 was given 900mg/kg of $AlCl_3$; Group 5 was given 200mg/kg ANS + 900mg/kg $AlCl_3$ and Group 6 was given 50mg/kg HNS extract + 900mg/kg $AlCl_3$. $AlCl_3$ significantly (p<0.05) decreased RBC count but showed a significant (p<0.05) increased in PCV, HB, MCH, and MCHC. Treatment with HNS and ANS reversed the effects of $AlCl_3$. But, group treated with HNS alone significantly (p<0.05) increased RBC count. Lymphocytes and WBC counts were significantly (p<0.05) decreased by $AlCl_3$, which was reversed with HNS. Treatment with either of the extract alone significantly (p<0.05) increased lymphocytes count. $AlCl_3$ significantly (p<0.05) decreased the level of MDA, and GSH as well as SOD and CAT activities. Treatment with ANS showed no significant (p<0.05) effect on MDA and GSH but significantly increased (p<0.05) catalase activity, whereas treatment with HNS significantly (p<0.05) decreased MDA level only. It can be concluded that extracts of *Nigella sativa* increased hematological parameters and alleviate oxidative stress in Wistar rats treated with $AlCl_3$

EFFECTS OF HYDROMETHANOL EXTRACTS OF *Garcinia kola* ON SOME BIOCHEMICAL PARAMETERS OF MALE WISTAR RATS.

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G*arcinia Kola* is commonly used in traditional medicine for the treatment of diverse ailments including coronary artery diseases. Thus, this study aims to determine the effect of hydromethanol (1:4) extracts of the pulp and seed coat of *Garcinia kola* on serum lipid profile and its antioxidant properties. The two forms were separately dried and blended to powder. Forty male wistar rats (8 per group) were assigned into Five (5) groups. Groups were treated thus: Group one; control. Group two; 100mg/kg pulp extract. Group three; 200mg/kg pulp extract. Group four; 100mg/kg seed coat extract. Group five; 200mg/kg seed coat extract; for 30 and 60 days duration. On treatment conclusion, blood was collected for the determination of lipid profile and antioxidant properties. The higher dose of the pulp and seed coat extracts significantly (P<0.05) increased the catalase level and superoxide dismutase enzyme activity, whereas, both the higher and lower doses of the seed coat extract caused a reduction in malondialdehyde level. The serum total cholesterol was significantly elevated by the higher dose of the pulp extract while the seed coat extract caused significantly increased high density lipoprotein cholesterol level and a reduction in the low density lipoprotein level. The two extracts demonstrated marked antioxidant effects. The seed coat of *Garcinia kola* may possess the potential to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The seed coat extract may also be useful in preventing coronary artery disease and other atherosclerotic problems.

SERUM LACTOGENIC HORMONES AND TOTAL MILK YIELD IN FEMALE LACTATING WISTAR RATS TREATED WITH EXTRACT OF *CITRULLUS LANATUS SEEDS*

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Around the world few herbs used for lactation purposes have not been scientifically evaluated but their traditional use, suggests and some beneficial effects. This study was designed to evaluate the lactogenic effect of *Citrullus lanatus* seeds (extract) in female lactating Wistar rats. Twenty apparently healthy female Wistar rats weighing between 160-200 g were randomly grouped into four (4) of five animals each (n=5) and treated orally for a period of ten (10) days, starting from day 3 after parturition. Group 1: Control (2 ml/kg) of distilled water; Group 2: Metoclopramide (5 mg/kg); Group 3: Extract (200 mg/kg); Group 4: Extract (400 mg/kg). Total milk yield was obtained from the difference between pre and post suckling pups weight. At the end of the treatment, rats (dams) were anaesthetized using diazepam and ketamine injection (75 and 25 mg/kg) respectively, and the blood samples obtained via cardiac puncture for biochemical analysis. There was a non-significant increase in serum prolactin level in all the treated groups when compared to control. Serum oxytocin level increased significant ($P< 0.05$) in groups 2, 3 and 4 compared to control. There was also a significant increase ($P< 0.05$) in serum oxytocin level in group 3 compared to group 2. Total milk yield increased significantly ($P< 0.05$) in the group 2 and 3 compared to control. In conclusion, *Citrullus lanatus* was found to increase oxytocin and milk yield in lactating Wistar rats.

ASSESSMENT OF LIPID PEROXIDATION AND SOME ANTIOXIDANT ENZYMES IN FEMALE LACTATING WISTAR RATS FOLLOWING ASCORBIC ACID AND α-TOCOPHEROL SUPPLEMENTATION

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Oxidative stress, an imbalance between the generation of reactive oxygen species/ reactive nitrogen species and antioxidant defence capacity of the body, is actively involved in the pathogenesis of diabetes and its complications. This study was designed to evaluate lipid peroxidation and antioxidant enzymes in female lactating rats. At parturition, the animals were randomly divided into five groups thus; Group I: (Normal control) was given normal animal feed and distilled water, (1 ml/kg), Group II: metoclopramide (5 mg/kg bw), Group III: 100 mg/kg bw of Vitamin E. Group IV: 100 mg/kg of Vitamin C, whereas Group V was treated with the co-administration of vitamin E and C. Administration was carried out orally for a period of ten (10) days at 06:00 hours daily and the animals were euthanized at the end of the experiment. Serum levels of MDA in group treated with ascorbic acid was significantly decreased ($P< 0.05$) compared to control, metoclopramide treated and α-Tocopherol treated groups. There was also a significant decrease in the grroups treated singly with vitamin C and α-Tocopherol when compared to the group co-administered with ascorbic acid and α-Tocopherol. However there was no statistically significant difference observed in the serum level of Superoxide dismutase (SOD) and Catalase (CAT). Serum level of Glutathione peroxidase (GPx) was statistically significant ($P< 0.05$) in the group administered α-Tocopherol compared to the control. In conclusion, ascorbic acid and α-Tocopherol administered singly was more efficient in alleviating lipid peroxidation than their co-administration.

DETERMINATION OF CORRECTED QT INTERVAL (QTC) AMONG PSYCHIATRIC PATIENTS AT DAWANAU PSYCHIATRIC HOSPITAL, KANO

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Psychiatric patients are often associated with electrocardiographic (ECG) abnormalities notable among which is prolonged QTc which in some cases are life threatening due to the effect of anti psychotic drugs they are placed on. This study aims to determine the QTc of patients on anti psychotic drugs and find out if there are abnormalities in the ECG tracings as documented in the literature. Anthropometric and clinical data were obtained from 323 participants attending Dawanau Psychiatric hospital between April to May 2017. Apparently normal individuals who were not taking anti psychotic drugs were used as controls. ECG was recorded using DEC G-03A 12 Lead ECG machine on all the patients lying supine on a couch. The mean age of the participants was 34.5±9.57 years with 182 (56%) being males and 141 (44%) females. Independent sample t-test among the subjects and controls for BMI, SBP and Heart rate were 21.4±4.4kg/m_2 and 21.57±3.57kg/m_2 (p=0.89), 111.42±16.67mmHg and 124.5±12.47mmHg (p=0.001), and 81.53±17.95 b/min and 73.37±12.03b/min(p=0.015) respectively. The mean corrected QT interval (QTc) was 413.51±30.09ms for the subjects and 391.47±18.38ms for the controls which is statistical significant (p=0.001). Nineteen (6%) of the subjects have prolonged QTc as against none of the controls, Correlation analysis within the subjects indicated no significant association between QTc and duration of anti-psychotic drugs treatment(r=1). It can be concluded that antipsychotic drugs do not affect QTc duration. It is therefore recommended that baseline ECG screening be carried out on all patients on anti-psychotic drugs to help identify those with salient features of myocardial diseases so as to institute concurrent treatment in order to minimize the risk of fatal complications.

LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES ASSESSMENT IN ALLOXAN MONOHYDRATE INDUCED HYPERGLYCAEMIC MALE WISTAR RATS FOLLOWING ORAL ADMINISTRATION OF THEOPHYLLINE AND GLIBENCLAMIDE

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The currently available anti-diabetic drugs are far from being satisfactory. Hence it is therefore imperative that the effects of other drugs like theophylline on blood glucose be studied.This study was designed to evaluate the effect of theophylline and glibenclamide treatment on lipid peroxidation (serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase, glutathione peroxidase and Catalase) in alloxan induced hyperglycaemic male Wistar rats. Thirty healthy male wistar rats weighing between 160-180g were grouped into five of six animals each (n=6) and treated for a period of fourteen days (14) after induction of hyperglycaemia using alloxan monohydrate. Group 1: (Normoglycaemic) Group 2: Diabetic control (DC), Group 3: Glibenclamide, 5mg/kg, Groups 4 and 5; theophylline 5mg/kg and 10mg/kg respectively. At the end of the fourteen (14) days, rats were anesthetized using ketamine and diazepam at 75 and 25 (mg/kg) respectively. Blood samples were taken from all treated groups for evaluation of serum MDA, SOD, GPx and CAT level. The result on serum MDA concentration was significantly decreased ($P< 0.05$) in glibenclamide treated group compared to diabetic control; 1.14±0.03 vs 1.32±0.06. Although a decrease was observed in the theophylline treated groups, the difference was however not statistically significant compared to diabetic control. There was also significant increase ($P< 0.05$) in serum SOD and CAT level in the glibenclamide and theophylline treated group (5 mg/kg) compared to DC; 2.02±0.04 and 1.92±0.24 vs 0.92±0.05 respectively for serum SOD and 53.20±0.58 and 52.80±1.07 vs 46.00±0.84 respectively for CAT. However, serum GPx increased significantly ($P< 0.05$) only in the theophylline treated groups compared to DC. In conclusion, Theophylline and Glibenclamide decreases lipid peroxidation while increasing serum antioxidant levels in alloxan induced hyperglycaemic male Wistar rats after 14 days oral administration

PREVALENCE OF MALARIA PARASITE (PLASMODIUM PARASITE) IN PATIENTS WITH SICKLE CELL ANAEMIA IN CRISIS STATE ATTENDING HAEMATOLOGY CLINICAL OF AHMADU BELLO UNIVERSITY TAECHING HOSPITAL ZARIA, NIGERIA.

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The sickle cell gene has a geographical distribution that is identical to plasmodium species hence its prevalence in the sub-Saharan Africa including Nigeria where malaria is endemic (Edington and Laing, 1957). The high incidence of the sickle cell gene in malaria holoendemic areais attributable to the protection it affords the bearer as explained by the concept of balance polymorphism (Serjeant andSerjeant,2001). Approximately, 300 million people world-wide are affected by malaria. It remains the major course of premature death, Abortion and still birth in the tropic and sub tropic, average of 1 – 1.5 million people die from it every year (WHO, 2003). Malaria is the most common cause of outpatient hospital visit in Nigeria and it consistently ranked among five most common cause of death for all ages (FMOH). This is a cross sectional study involving 30 consenting patients with SCA in crisis which was conducted in the haematology clinic of Ahmadu Bello University Teaching Hospital Zaria. All participants were interviewed using a structured questionnaire and examined clinically, consisting of 17 females (56.7%) and 13 males (43.3%). The ages ranges from 18 to 35. Malaria parasites were found in four (13.7%) patients with SCA in crisis. Females are less protected from malaria than males with incidence of 3 (13.7%) and 1 (7.7%) respectively. Males had more frequency of crisis while female had lower haematocrit level meaning that anaemia is worse in females. Blood transfusion and malaria are positively related. The prevalence of malaria in crisis is 13.7&% females are less protected from malaria than males. The have frequent crisis while the female have lower haematocrit level meaning that anaemia is worse in female. Blood transfusion and malaria are positively related.

COMPARATIVE STUDY OF OSMOTIC FRAGILITY TEST AND MALONDIALDEHYDE CONCENTRATION IN DEXAMETHASONE AND STREPTOZOTOCIN AS A MODEL OF TYPE 2 DIABETES IN HIGH FAT DIET FED RAT

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Different models have been experimented for easy generation of type 2 diabetes interval time. In order to further clarify the pathophysiology of this metabolic disease, this research was done to compare the effect of erythrocyte osmotic fragility (OFT) and malondialdehyde (MDA) concentrations in streptozotocin with high fat diet (STZ/HFD) and dexamethasone with high fat diet (DEXA/HFD) in Wister rats. For the study, 25 male rats of uniform weight and age were selected and divided into five groups, viz, Control group, dexamethazone (DEXA) 2ml/kg, high fat fed rats (HFD), (STZ 30mg/kg/HFD), and (DEXA 0.5ml/kg/HFD). At the end of the study, 5ml of blood of each animal was collected through cardiac puncture; 3ml was put in EDTA bottle and the remaining 2ml in plain tube for serum extraction which were subjected to OFT and MDA concentration analysis. Data obtained were analysed using ANOVA followed by Tukey's post-hoc test for descriptive analysis. The results showed significant increase in OFT for DEXA/HFD (38.20 ±0.86, 49.40 ±0.51, 68.60 ±2.71, 87.20 ±2.44 and 98.00 ±2.00 at 0.7%, 0.6%, 0.5%, 0.4% and 0.3% concentration of NaCl respectively) and STZ/HFD (37.40 ± 2.38, 57.00 ± 5.21, 73.20 ± 5.28, 82.40 ± 2.98, 91.40 ± 2.73 at 0.7%, 0.6%, 0.5%, 0.4% and 0.3% concentration of NaCl respectively) when compared with control group. Also for MDA serum concentration, both STZ (30.14 ± 1.63* nmol/ml) and DEXA (29.56 ± 0.30* nmol/ml)

showed statistical significant increase compared to control group. Conclusively, our findings indicate that rats treated with streptozotocin in combination with high fat diet and dexamethasone in combination with high fat diet are effective way to induce diabetes in Wister rat

PROTECTIVE EFFECT OF CAMEL MILK ON LIVER ENZYMES OF HIGH FAT DIET FED WISTAR RATS

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Camel milk exhibits antioxidant and insulin-like activities beneficial in diabetic mellitus. The study was aimed at investigating the protective effect of camel milk on activities of liver enzymes in diabetic Wistar rats. A total of 20 Wistar rats of both sexes used in the study were divided into four groups: Group I served as control; Group II, positive control; and Groups III and IV administered with camel milk orally at the dose rate of 2 and 4 ml/kg respectively for six weeks. Diabetes mellitus was induced in the rats by high fat diet, containing 10% of oil, 20% mill and 1% cholesterol. Aspartate aminotransferase activity was lower in diabetic rats administrated with camel milk at both 2 ml/kg (9.67 ± 1.93 IU) and at 4 ml/kg (7.97 ± 1.83 IU) than in diabetic group (90.2 ± 2.90 IU). Alanine aminotransferase activity was lower ($p<0.05$) in diabetic rats, administrated with camel milk at 4 ml/kg (6.48 ± 0.42 IU), compared to that of diabetic group (7.73 ± 0.98 IU). The concentration of Alkaline phosphate was lower ($p<0.05$) in diabetic rats, administrated with camel milk at the dose rate of 2 ml/kg (33.90 ± 3.00 IU) and 4 ml/kg (39.30 ± 8.00 IU) than that of the diabetic control (117.83 ± 15.70). Concentrations of total protein and albumin did not differ among the groups treated with camel milk. The result showed that camel milk reduced activities of the liver enzymes and may be beneficial in offering protection against diabetes mellitus. It was concluded that camel milk exerted protective effect against type 2 diabetes mellitus, and may be beneficial in its management.

CALABASH CHALK CHRONIC DIET CONSUMPTION ELEVATES ANXIETY AND PAIN PERCEPTION IN MICE

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Consumption of calabash chalk is a common practice in Nigeria as well as other parts of Africa, especially among pregnant women. Nevertheless, calabash chalk contains lead (Pb) and arsenic which are thought to be harmful to the brain and responsible for cognitive dysfunction. It is therefore conceivable that calabash chalk consumption may affect other neuronal activities in the body such as anxiety and pain. Therefore, this present research study investigated the effects of consumption of this form of pica on anxiety and pain perception in mice. Forty-five (45) Swiss white mice of mixed sex were randomly assigned into 3 groups of 15 mice each. Group 1 served as control, while groups 2 and 3 received low and high doses of calabash calk diets respectively. Feeding lasted for 30 days. Anxiety levels of the mice were assessed with the aid of elevated plus maze and light-dark transition box as well as elevated plus maze, while response to pain stimuli were studied using hot plate and formalin tests. The results showed that the calabash chalk diet-fed mice had significantly increased ($p< 0.05$) close arm duration and stretch attend posture compared to control. Pain perception was significantly increased in the calabash chalk diet-fed mice compared to control. Consumption of calabash chalk elevates anxiety and pain perception in mice. These actions may be as a result of its lead and arsenic content.

ASSESSING THE IMPACT OF RESTRAINT STRESS DURATION ON SOME SPERM INDICES AND OXIDATIVE STRESS BIOMARKERS OF MALE WISTAR RATS

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This experiment was aimed at determining the possible timing, which restraint stress induces oxidative damage and alters some sperm indices among Wistar rats. Animals were randomly divided into three groups of three animals each (n=3).Group I -normal control (undisturbed), Group II-(3 h stress) group, Group III- (6 h stress group). Restraint stress was induced by placing rats in specially constructed restraint meshes for both 3 and 6 hours (between 9.00-15.00 h) for 21 days. Testes homogenate were evaluated for Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH). 3 h stress group showed statistical ($P < 0.05$) decrease in sperm concentration and motility when compared to normal control group and 6 h stress group. However there was statistical ($P < 0.05$) increase in SOD and CAT in normal control groups when compared to stress groups. Restraint stress for both 3 and 6 h induced oxidative stress which might have led to decrease in sperm motility and concentration; however 3 h of stress induced more oxidative damage among male Wistar rats.

ABORTIFACIENT EFFECT OF AQUEOUS LEAF EXTRACT OF *AZADIRACHTA INDICA* (MELLACEAE) IN PREGNANT FEMALE RATS

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Rapid rise in population has caused serious problems in the economic and social growth of human development in countries like Nigeria, leads to poverty. About 80% of the world's population rely on herbal medicinal products as a primary source of medicine. The Abortifacient effect of *Azadirachta indica* was investigated by evaluating the serum estrogen and progesterone level in female pregnant Wistar rats. Female Wistar rats in their pro-estrous phase were cage with male in ratio 2:1. Rat exhibiting thick clump of spermatozoa in their vaginal smear were separated and that is day one of pregnancy.Total of twenty (20) female pregnant Wistar rats were randomly divided into four group of five rats each (n=5). Group I received (control) 1 ml/kg normal saline, Group II received 200 mg/kg Misoprostol, Groups III and IV received 250 mg/kg and 500 mg/kg of the aqueous extract of *Azadirachta indica* respectively. Misoprostol and aqueous extract of *Azadirachta indica* were administered orally on day 4th and day 5th of conception. The results showed that all rats in group I carry their pregnancy to term (delivered). The rats in groups II, III, and IV had their pregnancy aborted. Serum estrogen and progesterone level in aqueous extract treated groups when compared with control group showed a significant decrease. Also, the results for serum estrogen and progesterone in the aqueous extract treated groups when compared with Misoprostol group also showed no significant decrease. *Azadirachta indica* exhibit abortifacient effect as it caused abortion and decreases progesterone level which is the hormone that maintain pregnancy.

POSSIBLE ANTIOXIDANT EFFECTS OF *THEOBROMA CACAO L.* STEM BARK ON PHENYLHYDRAZINE-INDUCED ANEMIA IN WISTAR RATS

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Cocoa (*Theobroma cacao* L.) is a rich source of polyphenols and is been reported as having high antioxidant activity than teas and red wines. In hemolytic anemia, erythrocytes have a shortened life-span which is a part of the clinical syndrome associated with intoxwatayoication, where chemicals interact with sulfhydryl groups, inhibit enzymes, immune mechanisms, and the fragmentation of erythrocytes through the spleen or by unknown or poorly defined mechanisms. This study investigated the potential antioxidant effects of aqueous extract of *Theobroma cacao L.* stem bark on normal and phenylhydrazine (PHZ)-induced anaemic wistar rats. PHZ was used to induce anaemia intraperitoneally at a dosage of 60mg/kg (body weight) for two days. Forty five albino wistar rats weighing 126-224g were grouped randomly into 8 groups of 5 rats each. Group 1 served as normal control, received only water and feed, Group 2 (Anaemic control) was induced with anaemia without treatment, Group 3, 4, and 5 were induced with anaemia, received, 200mg/kg, 500mg/kg and 800mg/kg b.wt of the aqueous extract of *Theobroma cacao* stem bark respectively., while, groups 6, 7 and 8 were normal rats given 1000mg/kg, 3000mg/kg and 5000mg/kg b.wt of the extract for 28days. Some haematological parameters, enzymatic and non- enzymatic antioxidant activities were determined. Results obtained showed that high doses of the extract showed significant increase ($p<0.001$) in SOD activities in the normal rats,Catalase & MDA increased both groups. However, SOD levels reduced significantly in the treated anaemic rats. The increase in some of the antioxidant enzyme activities and concomitant reduction in severity of anaemia in the treated rats points to ameneorative effect of the antioxidant properties of Theobroma cacao L. on drug induce anaemia in wistar rats.

EVALUATION OF *OGOGORO* -INDUCED TESTICULAR TOXICITY IN ADULT MALE WISTAR RATS.

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Excessive alcohol consumption in beverages implicates several disease entities globally. The aim of this study is to evaluate the *Ogogoro* induced testicular toxicity in adult male wistar rats. Twenty (20) rats were divided into four groups of 5 rats (n=5) each. Group I: normal saline 2ml/kg, Groups II-IV: *Ogogoro* 3.5ml/kg, 7ml/kg, 14ml/kg respectively for sixty days. The result showed a significant decrease in serum testosterone and luteinizing hormone (LH) in groups III and IV respectively, when compared to the control. There was a significant decrease in sperm motility, increased number of dead spermatozoa in group IV when compared to the control ($P< 0.05$). Testicular weight and gonadosomatic index were significantly reduced in group IV treated rats. A significant increase ($P< 0.05$), in testicular MDA level in groups III and IV was found when compared to the control indicating increased lipid peroxidation. The testicular homogenate SOD, GPX and CAT activities in groups III and IV were significantly decreased when compared to the control. Ogogoro administered group IV showed irregular seminiferous tubules with epithelial sloughing. Ogogoro cause testicular toxicity via lipid peroxidation which could adversely affect male reproduction.

ASSESSMENT OF LIVER ENZYMES LEVELS IN TYPE 2 DIABETIC RABBITS TREATED WITH COMBINED SUPPLEMENTATION OF SYZYGIUM AROMATICUM (CLOVE) AND FERMENTED ZINGIBER OFFICINALE (GINGER) SUPPLEMENTS

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Monitoring vital organs functional status such as liver may leads to early detection of complications or adverse effects

of therapeutics. The aim was to evaluate the effects of clove and fermented ginger rhizome supplements on liver enzymes concentration in high fat diet induced type 2 diabetes in rabbits. High fat diet (SAF = 69% + Cholesterol = 1% + Ground nut meal = 20% + ground nut oil = 10%) was fed to rabbits for eleven weeks to ascertain diabetic animal model (DAM), thereafter, DAM were treated with supplements for six weeks. Twenty (20) male rabbits (5 weeks of age) divided into four groups (n=5) were used; Group I (Normal control) was treated with standard animal feed (SAF).Group II-IV (DAM groups) were treated as follows: Group II; treated with SAF only, Group III; treated with SAF + cholestran (0.26 g/kg) and Group IV; treated with SAF + clove + fermented ginger supplements, At the completion of treatments, animals were sacrifice and serum from blood samples was used for laboratory assessments of liver enzymes, data obtained were statistically analyzed using SPSS version 20.0. A significant ($P < 0.05$) increase in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in group II (diabetic animals fed on normal feed) were noticed, when compared to normal control group. While in group IV a significant ($P < 0.05$) decrease in serum AST when compared to diabetic rabbits group on normal feed was observed. In conclusion, combined clove and fermented ginger supplements reverses (down-regulates) elevated liver enzymes seen in high fat diet induced type 2 diabetic rabbits. Further work to validate these effects could facilitate the use of the supplement as a composite in formulating diet for type 2 diabetic patients.

EFFECT OF NICOTINAMIDE ON DEPRESSED MICE USING OPEN SPACE FORCED SWIM TEST MODEL OF DEPRESSION.

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Depression is a significant contributor to global burden of diseases and affects people of all communities across the world. The world health organization has ranked depression as the 4th leading cause of disability in the world and is projected to be the 2nd by the year 2020. In Nigeria, about 3.9% of the populations suffer from depression. Nicotinamide (NAM), has an anti-inflammatory property and can inhibit cytokines release, thus, having antidepressant potential. The aim of this study was to investigate the antidepressant effect of nicotinamide in depressed mice using open space forced swim test model of depression. Twenty-five mice of both sexes were randomly divided into five groups of five animals each; group 1 received normal saline 10 ml/kg, group II, III and IV received 25 mg/kg, 50mg/kg and100mg/kg of NAM respectively and group V received 20 mg/kg of fluoxetine. Tail suspension test (TST) was carried out on the animals before treatment which served as a baseline for depression. The animals were habituated to swimming for 4 days and on the fifth day, drug administration commenced which lasted for two weeks. The animals were only allowed to swim on the 1st, 4th, 7th, 10th and 14th day. Behavioural despair (immobility time) and locomotor activity (line crossing) of animals were assessed using tail suspension test, open space force swim test (OSFST) and open field test (OFT) respectively. The result obtained showed no significance. However, a statistically significant difference ($p < 0.05$) in 4th day between group two and five and also on the 7th day between group five when compared with group one and group two. There was no statistically significant difference in immobility of the Tail Suspension Test ($p > 0.05$) and line crossing in Open Field Test ($p > 0.05$) between the control and NAM treated groups. In conclusion, the result showed that NAM has no effect on the immobility time in depressed mice using OSFST.

MODULATORY ROLE OF VITAMINS A AND E ON MEMORY AND MOTOR FUNCTIONS OF CYANIDE INDUCED NEUROTOXICITY IN ADULT SWISS MICE

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Cyanide is a potent neurotoxic substance that can initiate series of intracellular reactions leading to oxidative stress. Vitamins A and E are antioxidants that have scavenging properties against free radicals and reactive oxygen species. This study was designed to evaluate effect of sublethal administration of potassium cyanide (KCN) and possible ameliorative role of vitamins A and E on sensorimotor functions and long term visuo-spatial learning and memory in adult Swiss mice. Thirty-five mice weighing between 18-22 g were used for the study. The animals were randomly divided into five groups (n = 7) and exposed to sublethal concentration of potassium cyanide (10% LD50; 1.5 mg/kg). KCN was administered orally while vitamin A (25 mg/kg) and vitamin E (50 mg/kg) were administered intra-peritoneally (IP) once daily for 28 days. KCN was administered first, followed after 10 minutes by vitamin A, and then vitamin E after 5 minutes. At the end of 28 days, mice were examined for signs of neuro-toxicity using wire grid, coat hanger and stationary beam test models. in the wire grid test, the latency to fall in weeks 2 and 4 were statistically significant ($p < 0.05$). In acquisition and retention, using elevated plus maze (EPM), KCN treated group recorded high transfer latencies in seconds (50.40±1.72 secs) and (57.60±0.93 secs) as compared to group IV (29.40±0.68 secs; 5.60±0.60 secs). It was concluded that KCN affects motor coordination and memory in mice, while treatment with antioxidant vitamins A and E ameliorated these deficits.

AMELIORATIVE EFFECT OF EUGENOL ON ALUMINIUM CHLORIDE-INDUCED NEPHROTOXICITY IN WISTAR RATS

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Kidneys are essential to the urinary system and play critical role in homeostasis. Eugenol is known for its medicinal value and is an established antioxidant present in plants like, clove, basil and nutmeg. This study histologically and biochemically assessed the ameliorative effect of Eugenol on aluminium chloride ($AlCl_3$)-induced nephrototoxicity in Wistar rats. Thirty Wistar rats of bith sexes (95 - 110 g) were divided into six groups (A – F) of five rats each. Group A served as the control and was administered distilled water (2 ml/kg), Group B was administered $AlCl_3$ (100 mg/kg) only. Groups C - F were administered Eugenol (150 mg/kg, 225 mg/kg and 300 mg/kg, respectively), and Silymarin (100mg/kg), before $AlCl_3$ (100 mg/kg). All the Administrations were via oral Gavage for the duration of three weeks. The Ameliorative effect of Eugenol was assessed using light microscopic examination of routinely (H and E) stained kidney sections and biochemical analysis of kidney electrolytes (Na^+, K^+, Cl^- and HCO_3^-) and Urea. Results revealed the distortion of the histoarchitecture of the renal parenchyma and elevated levels of kidney electrolytes in $AlCl_3$-treated group when compared to the control ($p>0.05$) and Eugenol-treated ($p<0.05$) groups. However, administration of Eugenol ameliorated $AlCl_3$-induced kidney damage by preservation of the kidney histoarchitecture and, decreased ($p<0.05$) serum kidney electrolytes levels. Eugenol ameliorative activity was comparable with that of Silymarin, especially at dose 225 mg/kg. Eugenol possesses nephroprotective potentials against heavy metal-induced acute nephrototoxicity in Wistar rats.

WAIST TO HEIGHT RATIO CORRELATES NEGETIVELY WITH DURATION OF MENSTRUAL BLEEDING AMONG FEMALE STUDENTS OF BAYERO UNIVERSITY, KANO.

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Obesity is associated with menstrual irregularity, sub fertility and infertility presumably due to obesity-associated increases in peripheral estrogen production by the adipose tissue. It has been suggested that accumulation of fat centrally may be more associated with menstrual irregularities than overall adiposity such as BMI. This study therefore aimed at assessing the relationship between indices of obesity and menstrual cycle characteristics among female students of Bayero University, Kano. Using self-administered questionnaire the menstrual characteristic of 283 students between the ages 16 to 26 years, were studied. Body mass index (BMI) was calculated as the subject's weight (kg) divided by the square of the subject's height (m^2) and waist-height ratio (WHtR) was calculated as waist circumference divided by height. The data was analyzed using IBM SPSS Statistics for Windows, version 22.0. Quantitative data was presented as mean ± SD, and qualitative data was presented using percentages and frequency. $p\leq0.05$ was considered statistically significant. Most of the respondents 184 (65%) had their weight within normal, ther mean age at menarche was 13.55±1.50; mean length of menstrual cycle was 26.68±3.51; and the mean duration of menstrual bleeding was 5.18±1.24. A statistically significant difference between the body mass index and duration of menstrual bleeding was found. The study also found a significant negative correlation between waist to height ratio (WHtR) and duration of menstrual bleeding.

PROTECTIVE EFFECT OF L-ARGININE CO-ADMINISTRATION WITH HIGH-FAT DIET ON ERYTHROCYTE OSMOTIC FRAGILITY AND MALONDIALDEHYDE CONCENTRATION IN MALE WISTAR RATS

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The excessive consumption of high-fat diet is associated with an increased incidence of obesity, resulting in oxidative stress and lipid peroxidation. This study was designed to evaluate the protective effect of L-arginine co-administration with high-fat diet on erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration in male Wistar rats. Thirty (30) adult Wistar rats used for the study were divided into six groups of five rats each: Group I: (Normal Control) Received distilled water (1 ml/kg) with normal feed. Group II: (Diabetic control) Received high-fat diet (HFD) only. Group III: Received NFD + 200 mg/kg of L-arginine. Group IV: Received NFD + 400 mg/kg of L-arginine. Group V: Received HFD + 200 mg/kg L-arginine. Group VI: Received HFD + 400 mg/kg of L-arginine. The result shows significant decrease ($P < 0.05$) EOF only in the 400 mg/kg Arg+HFD group (84.0±2.28%) (55.4±2.0%) as compared to normal control (90.6±1.72%) (60.4±2.02%) and HFD-only group (92.4±1.60%) (62.8±0.92%) at 0.3% and 0.4% respectively. The result on MDA concentration showed a non-significant increase ($P > 0.05$) in all the treated groups when compared to normal group. The highest MDA concentration was observed in the group treated with high-fat diet only (0.96 ± 0.10 µMol/L), when compared to normal (0.72 ± 0.10 µMol/L). It was concluded that HFD increased haemolysis and MDA concentration in Wistar rat, and this effect was ameliorated by L-Arginine administration.

EVALUATION OF THE AQUEOUS EXTRACT OF *Datura stramonium* ON OXIDATIVE STRESS BIOMARKERS OF *Plasmodium berghei*-INFECTED MICE

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Malaria is one of the major infectious diseases in Africa, especially in Nigeria. The aim of this study was to evaluate the effect of an aqueous extract of *Datura stramonium* on some oxidative stress biomarkers of *Plasmodium berghei*-infected mice. The aqueous extract of the leaves at 250, 500 and 1000 mg/kg body weight/day dose levels were used to treat the test groups after infection for four days (Adia *et al.*, 2014), while a standard antimalarial drug, Cloroquine, at a dose of 25 mg/kg body weight was administered on the positive control group. The negative control group was left untreated. The variation in the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) in the different groups were observed throughout the study. The crude extract was screened for its phytochemical composition. Results showed a marked decrease in GSH level and activity of SOD and GSH after infection signifying oxidative stress. However, there was a significant rise (P<0.05) in SOD and GSH levels in the group treated with 500 mg//kg body weight of *D. stramonium* as compared to other treatment groups. More so, the activity of catalase across groups treated with *D. stramonium* also showed considerable increase. The screening for the phytochemical composition of the crude extract showed the presence of alkaloids, flavonoids, and saponins, while tannins and anthraquinones were absent. The findings of this study showed that *Datura stramonium* may be used as an antimalarial regimen, as its' application does alleviate oxidative stress as seen in the biomarkers determined.

ANTIDIARRHOEAL ACTIVITY OF METHANOL LEAF EXTRACT OF *HYPTIS SUAVEOLENS* (L.) POIT (BUSH MINT).

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Diarrhoeal disease is prevalent in tropical countries with poor hygiene and low standard of living. Diarrhoea is a leading cause of malnutrition and death due to its dehydrating effect and loss of electrolytes. *Hyptis suaveolens* has been used traditionally around the world for various ailments and diseases. The plant is also consumed as foods in some communities. The phytochemical analysis and acute toxicity test of the methanol leaf extract were evaluated. Antidiarrhoeal activity of the leaf extract was evaluated using castor oil and magnesium sulphate induced diarrhoea in rats and chicks at doses of 250 mg/kg, 350 mg/kg and 500 mg/kg body weight orally. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins and triterpenes. The oral LD_{50} of the extract was above 5000 mg/kg in both rats and chicks. The methanol leaf extract of *Hyptis suaveolens* at tested doses significantly (p<0.05) reduced the frequency and weight of stool in castor oil and magnesium sulphate - induced diarrhoea in rats and chicks in a dose and time dependent manner compared to loperamide (2 mg/ml). It can be concluded that *Hyptis suaveolens* has antidiarrhoeal activity in rats and chicks which may be attributed to some of the phytochemical constituents.

POLYUNSATURATED FATTY ACID METABOLISM AND ANTIOXIDANT RESPONSES IN ADULT LACTATING WISTAR RATS FOLLOWING ORAL ADMINISTRATION OF MONOSODIUM L-GLUTAMATE (MSG)

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Several studies have reported toxic effects of MSG on the reproductive system however; the concept of oxidative shielding suggests that during reproductive exercises the likes of lactation, mothers preemptively decrease their levels of oxidative stress for the sole of purpose of shielding or protecting their offspring from detrimental effects of damaged molecules like oxidized fatty acids. Hence this study was designed to investigate the effect of MSG on oxidative status of Wistar rats during lactation. Following birth, the dams were divided into four groups of six (n=6) rats each having six pups. Oral administration of MSG lasted for 2 weeks as follows: Group 1: Distilled water (2 ml/kg), Group 2: Metoclopramide (5 mg/kg), Group 3: MSG 1850 mg/kg and Group 4: MSG 3700 mg/kg. The result of serum malondialdehyde (MDA) concentration (Umol/L) was lower significantly (P< 0.05) in all the MSG administered groups compared to control and metoclopramide (5 mg/kg) treated. Serum superoxide dismutase (SOD) (Umol/mg) was significantly higher (P< 0.05) in MSG administered groups compared to metoclopramide (5 mg/kg). Serum level of glutathione (GSH) (Umol/mg protein) was significantly higher (P< 0.05) in MSG 3700 mg/kg administered group compared to control, MSG 1850 mg/kg and metoclopramide (5 mg/kg). Although serum catalase (CAT) level was decreased in both MSG and metoclopramide groups compared to control, the difference was however only statistically significant (P< 0.05) in 3700 mg/kg group. Oral administration of MSG increased serum SOD and GSH level with a significant decrease in MDA concentration in lactating Wistar rats.

INFLUENCE OF VARYING DEGREE OF WOOD DUST EXPOSURE ON PULMONARY FUNCTION AND RESPIRATORY SYMPTOMS AMONG WOOD WORKERS IN KANO, NORTH WESTERN NIGERIA.

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One of the major occupation-related health challenges encountered by wood workers is respiratory disorder, which usually results from breathing in noxious or toxic chemicals such as wood dust. The aim of this study is to

evaluate the respiratory functions and symptoms among wood workers exposed to varying degrees of wood dust in Kano, Nigeria. This descriptive cross-sectional study was carried out among 370 randomly selected wood workers in Kano wood market. Lung function test was performed, while semi-structured interviewer administered questionnaire was used to rate respiratory symptoms. The study demonstrated that there is low percentage predicted force expiratory volume at one minute ($PPFEV_1$) and percentage predicted ratio of FEV1 and FVC, whereas, the percentage predicted forced vital capacity (PPFVC) of the respondents across all age groups remained unchanged. A statistically significant association existed between exposure to wood dust and respiratory symptoms (χ_2 = 16.2, df = 1, p = 0.001), thereby contributing to the observed manifestation of respiratory symptoms such as chronic cough, corrhiza, breathlessness and wheezing among 61% of wood dust exposed workers. Similarly, a negative correlation was observed between degree of exposure to the hazards and lung function of the workers (r = -0.655, P-Value = 0.0001).

COMPARATIVE EFFECT OF *Nigella sativa* SEED OIL AND ZINC GLUCONATE ON ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE AND GASTRIC SECRETION IN WISTAR RATS

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A wide spread search has been launched to identify new anti-ulcer therapies from natural Sources, with minimal side effects. The aim of the study was to compare the effects of *Nigella sativa* seed oil and zinc gluconate on ethanol-induced gastric mucosal damage and Secretion in Wistar rats. A total of 50 male rats, weighing between 150-200g were used for the study. The animals were then sub-divided into two sub-groups of 25 rats each for gastric mucosal damage and gastric secretion studies respectively. Each of the sub-groups were divided into 5 groups of 5 rats each and treated with distilled water (10ml/kg), absolute ethanol (1ml/kg), *Nigella sativa oil* (5ml/kg), zinc gluconate (50mg/kg) and zinc gluconate (50mg/kg) plus *Nigella sativa* oil (5ml/kg) respectively. The results of the study revealed the normal architecture of gastric mucosa for the control group with ulcer index of 0.0 ± 0.0 mm. The ethanol-treated group showed severe necrosis-of gastric epithelium, with ulcer index of 10.25 ± 0.85 mm as compared to the control. The *Nigella sativa* oil, zinc gluconate and *Nigella sativa* plus zinc gluconate treated groups have demonstrated significant decrease in the ulcer indices of 5.50 ± 1.04 mm, 4.75 ± 0.25mm and 3.75 ± 1.44mm respectively as compared to the control with 0.0 ± 0.0 mm. On the other hand the groups also showed preventive indices of 48.8%, 53.6% and 63.4% respectively when compared to those of the control 100% and ethanol treated group with no protection. In conclusion, *Nigella sativa* and zinc gluconate conferred protection to gastric mucosa but the combination of the two produced an additive effect.

LAURIC ACID ALLEVIATES INFLAMMATION AND STRUCTURAL CHANGES IN THE LUNGS OF TYPE II DIABETIC MALE WISTAR RATS

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Diabetic complication in the lungs is characterized by infiltration of inflammatory mediators in the lungs and structural alteration of the lung parenchyma. This study was designed to evaluate the effect of lauric acid on leucocytes infiltration in **bronchoalveolar lavage fluid** (BALF), concentration of TNF-α and lung histology of type II diabetic male Wistar rats. Type II diabetes was induced using high fat diet/40 mg/Kg streptozotocin along with 20% fructose solution. A total of thirty-five male Wistar rats were randomly divided into seven groups of five rats each as follows: Group **I** was normoglycemic Wistar rats administered 1ml/Kg distilled water, and served as normal control. Group II was normoglycemic Wistar rats administered 125 mg/Kg lauric acid. Group III was diabetic Wistar rats administered 1ml/Kg tween 80, and served as diabetic control. Groups IV, V, VI and VII were diabetic Wistar rats treated with 125 mg/Kg, 250 mg/Kg, 500 mg/Kg lauric acid and 100 mg/Kg metformin orally respectively. The results obtained, showed a significant ($P \leq 0.05$) increase in total and differential white blood cell count in blood and BALF of the diabetic rats, however, it was significantly decreased after treatment with lauric acid. The concentration of TNF-α was significantly higher in the lungs of diabetic rats, but treatment with lauric acid has reduced it significantly. Lauric acid also reversed the reduced alveolar spaces in diabetic lungs. It can be concluded that lauric acid reduced inflammation and reversed the histoarchitectural alterations in the lungs of type II diabetic male Wistar rats.

TAURINE TREATMENT MODULATES HEMATOLOGICAL INFLAMMATORY MARKERS IN PAPAIN INDUCED ARTHRITIS IN ADULT FEMALE WISTAR RAT.

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Osteoarthritis (OA) is one of the most prevalent and debilitating joint disease associated with reduced quality of life and increased healthcare costs. Available medical therapies, including traditional analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) for OA are ineffective at slowing down the disease progression, but rather alleviate the symptoms by reducing pain. Additionally, their chronic use has been linked to number of deleterious side effects. This study aimed to evaluate the effect of taurine on some inflammatory hematological parameters in papain induced osteoarthritis in adult female

Wistar rats. Thirty female rats were acclimatized and randomly divided into six (6) groups: control group (G1), disease control group (G2), standard control group (G3), taurine treated groups (G4, G5 and G6). G1 received distilled water 1ml/Kg, G2 received 1ml/Kg of distilled water, G3 received Diclofenac sodium 5mg/Kg, while taurine treated groups G4, G5, and G6 received 100mg/Kg, 200mg/Kg and 400mg/Kg of taurine respectively. OA was induced by intra-articular injection of papain into the right knee joint of the rats on days 1, 4 and 7 before the commencement of the treatment. The parameters checked include; Erythrocyte sedimentation rate (ESR), Neutrophil lymphocyte ratio (NLR), Platelet lymphocyte ratio (PLR) and diameter of inflammation on the knee joint. There was a significant ($P<0.05$) reduction in ESR, NLR, PLR and diameter of inflammation induced in the knee joint in the taurine treated groups as compared to the disease control group. In conclusion taurine was able to decrease inflammatory hematological parameters in papain induced arthritis in female Wistar rats.

PROTECTIVE EFFECT OF CO-ADMINISTRATION OF VITAMINS C AND E ON RESERPINE-INDUCED MOTOR IMPAIRMENT IN MICE

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The conventional treatments for Parkinson's disease, the most common movement disorder worldwide, have not been able to halt its progression, hence, newer approaches targeting its pathogenesis are being explored. We investigated the effect of combining vitamins C and E on reserpine-induced motor impairment in mice. Twenty-five mice were assigned into 5 groups. Group I (control) received distilled water only while the other groups received reserpine 0.1mg/kg intraperitoneally on alternate days. In addition, Group III (vitamin E group) received vitamin E 200 mg/kg/day, group IV (vitamin C group) received vitamin C 250 mg/kg/day and group V (co-administered group) received both vitamins orally. Group II (reserpine group) received nothing in addition to reserpine. All drugs were given concurrently for 28 days. Neurobehavioral assessment was performed using beam walking and open field tests. Results were expressed as mean ± SEM and values at $p < 0.05$ were considered significant. The increase in number of foot slips (3.60 ± 0.68) as well as the time taken to reach the safe platform (36.60 ± 5.78 s) observed in the reserpine group were significantly decreased in the co-administered group (0.25 ± 0.25 and 3.00±0.41s respectively). The transfer latency was significantly decreased (10.33 ± 1.45 s) with a marked increase in the number of lines crossed (56.00 ± 13.53) in the co-administered group compared to reserpine group (214.00 ± 64.16 s and 4.3 ± 1.67 respectively). The co-administration of vitamins C and E confers a significant neuroprotection against reserpine-induced motor impairment in mice.

INVESTIGATION OF THE EFFECTS OF AQUEOUS EXTRACT OF *Piper guineense* (ASHANTI PEPPER) SEED ON INDICES OF HEPATIC FUNCTIONS

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Piper guineense seed is widely consumed in some part of West Africa for its nutritional and medicinal properties. This study investigated the effect of its aqueous extract on indices of hepatic functions. Twenty Wistar rats were purchased and divided into four groups of five per group. They were allowed access to feed and water for two weeks. Different concentrations of the extract were administered to the three experimental groups: 100 mg/Kg, 200mg/Kg, 400 mg/Kg, while control group were given feed and water. The feeding lasted for 21 days. The result showed that liver enzymes Alanine transaminase, aspartate aminotransferase, Alkaline phosphatase showed no significant difference ($p>0.05$) between the experimental groups and control groups. Total protein and Globulin of the experimental groups were significantly ($p<0.05$) lower when compared to the control group but albumin did not. The group that received the highest dose of the extract had significantly lower total bilirubin when compared with the control group ($p<0.05$) while conjugated bilirubin did not. Histology showed that only high dose of *Piper guineense* distorted the normal histo-architecture of the liver. Thus, moderate consumption of *Piper guineense* seeds is recommended; high dose may be harmful to the liver.

EFFECT OF ETHANOL EXTRACT OF *ALLIUM SATIVUM* ON HEMATOLOGICAL PARAMETERS IN E *COLI* INDUCED SEPSIS IN WISTAR RAT

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One of the agents of sepsis include the gram negative E. coli. Over the years of study, spices, such as Allium sativum, seems to combat infection. The aim of this work is to determine the effect of ethanolic extract of allium sativum on hematological parameters and osmotic fragility of red blood cells of Wistar during E. coli induced sepsis. Thrity-five (35) rats were used in this study and were grouped into 7 groups (n=5). Except for group 1 (normal control), all groups were induced with sepsis by E coli interperitoneal injection. Successful induction was confirmed after 5 days. All groups, except group 1 and group 2, the negative control group, were treated for 14 days. The remaining groups were treated with 400mg/kg/day of hydrochloride ciprofloxacin; HC (group 3; positive control), 200mg of extract/kg/day (group 4), 400mg of extract/kg/day (group 5), 200mg of extract /kg/day with HC (group 6) and 400mg of extract/kg/day with HC (group 7). After 14 days, the hematological parameters and RBC osmotic fragility test were obtained and analyzed ($P<0.05$ and $P<0.01$). Though the extract seems to boost the hematocrit and hemoglobin concentration, it also made them more fragile especially in

the presence of Cirpofloxacin. These could lead to hemolytic anemia. Garlic should be used with hematinics to treat infection. Combined therapy of Garlic and standard drug should not also be encouraged as it worsens the anemia caused by standard drug.

ELECTROCARDIOGRAM PATTERN AND BLOOD PRESSURE IN ADULT SICKLE CELL ANAEMIA PATIENTS IN SOKOTO, NIGERIA

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The present study was aimed at determining the electrocardiographic (ECG) pattern of sickle cell anaemia (SCA) in adults attending the sickle cell clinic at the Haematology Outpatient Unit of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. A total of 148 subjects comprising 55 SCA patients (in crisis), 53 SCA patients (in the steady state) and 40 normal control groups were studied. Following ethical approval, ECG and blood pressure (BP) were measured using standard methods[1]. Electrodes were positioned as recommended by the American Heart Association[2] and BP with the aid of a mercury sphygmomanometer. Patients had significantly (P<0.001) lower systolic BP in the steady state (108±1.6mmHg) and in crisis (105±1.7mmHg) compared to controls (122.9±1.7mmHg). The diastolic BP of patients in the steady state (63.0±1.9mmHg) was significantly (P<0.001) lower than in the control group (73.1±1.7mmHg) but did not differ from the value recorded in patients in crisis (65.5±1.6mmHg). There was no significant difference in the heart rate, P wave duration, QTc and PR interval of patients and control. However, QRS duration was significantly (P<0.0001) lower in SCA patients in the steady state (54.2±2.2ms) and in crisis (53.3±2.8ms) than in controls (72.1±3.0ms). In conclusion, this study shows that the SCA patients had lower BP and QRS duration than healthy controls.

DIURNAL FLUCTUATIONS IN MECHANICAL, THERMAL AND CHEMICAL PAIN THRESHOLDS IN MALE AND FEMALE WISTAR RATS DURING HARMATTAN SEASON IN NORTH WESTERN NIGERIA

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This study investigated the diurnal fluctuation of mechanical, thermal, chemical pain in the light phase and dark phase and also considering their sex based differences during the harmattan season conducted in the North-West Nigeria. The work was carried out in two phases (light and dark phase). Light phase done at 07:00h-10:00h, while Dark phase at 19:00h- 22:00h. The rats were made to undergo the experimental pain assessment (Mechanical, thermal and chemical), also, the temperature and the relative humidity of the day were checked and recorded hourly. Animals were randomly grouped in to two phases, the light phase group and the dark phase group. Animals in the light phase group were grouped in to male and female groups. Animals in the male group were further sub grouped in to three groups of 5 animals each for the three different pain threshold assessment test. Mechanical and thermal pain threshold showed no statistically significant difference between the light phase and the dark phase ($p > 0.05$). Chemical pain threshold was significantly higher in the dark phase when compared to the light phase ($p< 0.05$).

IMPAIRED SPERM PARAMETERS IN STREPTOZOCIN INDUCED DIABETIC MALE WISTAR RATS AND THE EFFECTS OF LAURIC ACID

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Diabetes mellitus is associated with a decline in reproductive function. Studies have shown that coconut oil (CO) possesses anti-diabetic properties and ameliorative effects on impaired sperm parameters. Lauric acid (LA) is the most abundant constituent of CO. Thus, this study sought to investigate the effects of LA on sperm parameters in diabetic male Wistar rats. The animals, divided into 6 groups of five (n=5), received treatments orally for 4 weeks as follows: Group I: distilled water (1ml/Kg), Group II: Diabetic untreated, Group III: Diabetic + LA (90 mg/Kg), Group IV: Diabetic + LA (180 mg/Kg), Group V: Diabetic + LA (360 mg/Kg) and Group VI: Diabetic + CO (1.42 ml/Kg). The results show a significant decline ($P < 0.05$) in the concentration, motility, normal morphology and viability of sperm cells in diabetic untreated rats compared to the normal control rats. In diabetic rats treated with CO; sperm concentration, percentages of motile, normal and viable sperm cells were significantly higher ($P < 0.05$) compared to the diabetic untreated rats. Compared to the diabetic untreated rats; only the percentage of progressive motile sperm cells in diabetic rats treated with 90 mg/Kg LA and the percentage of normal sperm cells in diabetic rats treated with 360 mg/Kg LA respectively, were significantly higher ($P < 0.05$). Furthermore, the impacts of the above doses of LA were significantly lower ($P < 0.05$) compared to the treatment with CO. Thus, impaired sperm parameters in diabetic rats were not completely alleviated by lauric acid.

EFFECT OF STEP AEROBICS ON BLOOD GLUCOSE LEVEL AND CARDIORESPIRATORY PARAMETERS OF OVERWEIGHT ADULTS IN VOM, PLATEAU STATE, NIGERIA.

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This study was aimed at determining the effect of step aerobics training on blood glucose and cardiorespiratory parameters of overweight adults in Vom, Plateau State, Nigeria. Twenty (20) overweight adults participants were selected using the purposive sampling technique from Vom, Plateau State, Nigeria. Participants were trained for 8weeks with a frequency of 3 days per week and a duration of 48minutes with a moderate intensity of HR max of between 50-65%. Blood Glucose Level (BGL), Resting Heart Rate (RHR), Peak Expiratory Flow Rate (PEFR) and forced expiratory volume in one second (FEV_1) were taken at pre training and post training(after 8 weeks of step aerobics training) respectively. Results showed that Step aerobics training significantly reduced the BGL of overweight adults ($P<0.05$), caused no reduction on the RHR of overweight adults ($p>0.05$) and increased the PEFR and FEV_1 of overweight adults ($P<0.05$). The effect of step aerobics on the overweight adults has proven to be generally positive on the basis of these findings, therefore Step aerobics should be publicized in fitness and wellness centers as a mode of training as it has shown evidence of metabolic and cardiorespiratory adaptations in overweight adults by causing a reduction in blood glucose level, increasing peak expiratory flow rate and forced expiratory volume in one second.

EFFECT OF LACTATIONAL EXPOSURE TO FLAVONOID-RICH EXTRACT OF *HIBISCUS SABDARIFFA* ON THE ONSET OF PUBERTY AND REPRODUCTIVE HORMONE PROFILE IN THE OFFSPRING OF ALBINO RATS

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The effect of lactational exposure to flavonoid-rich extract of *Hibiscus sabdariffa* from birth to postnatal (PND) 42 was investigated in Albino rats. Sixteen in-bred pregnant rats were randomly assigned to four groups (A, B, C, D) of four rats each and were given rat chow and water adlibitum. From birth to PND 21 (weaning), all groups had normal rat chow and water adlibitum. The dams of the test groups were administered with rich extract of flavonoid via oral gavage daily, 10mg/kg body weight (B), 20mg/kg body weight (C) and 50mg/kg body weight (D), throughout the entire period of lactation (PND 0–– PND 21). At PND 21, the pups were designated into four groups according to their dams. Water and fluid intake was measured daily. From PND 21 pups were monitored daily for Balano-preputial separation and Vaginal opening. At PND 42, blood sample was collected by occulo-puncture for the assay of reproductive hormones of both male and female pups. Results from this study showed a significant ($P>0.05$) delay on the onset of puberty, increase in weight and Body Mass Index (BMI) of the test rats as compared with the control. Also, there was a significant ($P>0.05$) decrease in circulating plasma levels of LH, FSH and Testosterone (male), while there was a significant increase ($P<0.05$) in the circulating plasma level of Estradiol (female) of these pups. Flavonoid was seen to delay the onset of puberty and decreased circulating levels of reproductive hormones.

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

22-27 September, 2019, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

OMEGA - 3 FATTY ACIDS MITIGATE OXIDATIVE AND INFLAMMATORY EVENTS IN LEAD - TREATED RATS

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Lead remains a constant threat to our environment because of its non-biodegradable nature (Olayaki *et al*., 2018). Therefore, the study investigated the effects of omega-3 fatty acids (N-3) in lead-treated rats. Twenty male Wistar rats of five animals per group were used for this study. The control group received saline (0.1ml, *po*, daily) during the four weeks duration of the experiment, while group 2 received lead chloride ($Pbcl_2$) and saline during the first and last two weeks of the experiment respectively. Groups 3 and 4 were administered lead during the first two weeks; afterwards, they were treated with N-3 during the subsequent two weeks. $Pbcl_2$ was administered at 50mg/kg bw/day(*po*), while N-3 were administered at a low and high dose of 100 and 300mg/kg b.w./day(*po*) respectively. The results showed that there were significant elevations in the plasma levels of TNF-a, IL-6, CRP and MDA, and significant decreases in SOD, catalase and total antioxidant capacity (TAC) in the lead untreated group (Kim *et al*., 2007). Post-treatment with N-3 after lead exposure caused significant diminutions in TNF-a, IL-6, CRP, ROS, and MDA, and significant increases in catalase, SOD and TAC, relative to lead untreated group. The low dose of N-3 had more significant effects on TNF-a, IL-6, CRP, MDA and TAC, compared to the high dose. However, the latter demonstrated more significant effects on catalase, SOD and ROS. The study concluded that dietary supplementation with N-3, preferably at a low dose, mitigate the adverse effects of lead via antioxidative and anti-inflammatory mechanisms

MODULATORY ROLE OF SELENIUM YEAST ON OXIDATIVE STRESS BIOMARKERS OF CHOLESTEROL DIET INDUCED TYPE 2 DIABETES MELLITUS IN WISTAR RATS

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Selenium is an antioxidant that prevents oxygen radical from damaging cells from chronic diseases that can develop from cell injury and inflammation such as diabetes mellitus. The aim of the study is to investigate the possible protective effect of selenium yeast on oxidative stress biomarkers of cholesterol diet induced type-2 diabetes mellitus in rats. Twenty male wistar rats were divided in to four groups of five animals each: Group 1: (Negative control) received standard animal feed only, Group 2: received cholesterol diet (CD) only, Group 3: received CD and 0.1 mg/kg selenium yeast orally, Group 4: Received CD and 0.2 mg/kg selenium yeast orally for six weeks. At the end of the study period, the animals were sacrificed and the serum samples were collected and evaluated for estimation of blood glucose levels and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The results showed a significant ($P < 0.05$) decrease in blood glucose level in the groups co-administered CD and selenium yeast when compared to CD group only. Antioxidant enzymes status recorded significant ($P < 0.05$) decrease in SOD, CAT and GPx activities in CD and selenium yeast administered when compared to CD group only. In Conclusion, Selenium yeast administrations prevent free radical formations which are potent inducers of diabetes mellitus.

AGE-DEPENDENT CHANGES IN VASCULAR REACTIVITY IS INDUCED BY ARGINASE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Arginase activity reduces substrate (L-arginine) availability for nitric oxide formation while enhanced vascular arginase activity contributes to endothelial dysfunction in Dahl-S rats with salt-induced hypertension. There isa paucity of information on vascular arginase activity of aged normotensive and spontaneously hypertensive rats (SHR). This study tests the hypothesis that the age-dependent vascular endothelial dysfunction in thoracic aortafrom SHRs is mediated by Arginase. Acetylcholine (ACH) [10^{-9}–$3x10^{-5}$M] - induced concentration-dependent relaxation responses of thoracic aortic rings from young and old SHR and control ratswere evaluated, using wire myography, in the absence and following incubation for 30 minutes with L-Arginase (0.5U/ML). Endothelium-independent relaxation was also evaluated using sodium nitroprusside (SNP) [10^{-12} – $3x10^{-}$

$^{-5}$ M]. Vessels were pre-constricted with 10^{-6}M phenylephrine. Data are expressed as mean ± S.E.M. of 6 rats per group; statistical differences were calculated using Student's t-test and two-way ANOVA with repeated measures followed by Bonferroni post hoc test. Significance was set at $p<0.05$. ACH and SNP-induced relaxation responses were significantly decreased in aortic rings from old SHRs incubated with L-arginase compared with the rings without L-arginase ($p< 0.0001$) but were unchanged in the young SHRs. ACH and SNP relaxation responses were also unchanged in aortic rings from old and young control rats, in the absence or presence of L-Arginase [0.5U/ML]. Results suggest that arginase promotes vascular endothelial dysfunction in the old SHR as well as vascular smooth muscle dysfunction.

EFFECT OF HEXANE LEAF EXTRACT OF *LAUNAEA TARAXACIFOLIA*, RESVERATROL AND THEIR COMBINATION ON SERUM PROLACTIN AND OXYTOCIN LEVELS OF LACTATING FEMALE WISTAR RATS

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Lactogenesis, a neuro-endocrine event, is a complex neurophysiological process that involves interaction of a number of physical and emotional factors along with the action of multiple hormones, mainly prolactin. The aim of this study was to evaluate the comparative effects of *Launaea taraxacifolia* and resveratrol on serum prolactin (Prl) and oxytocin (Oxt) levels of lactating female Wistar rats. Twenty-five mature nulliparous female rats weighing 150-200 g were bred for the study. Following parturition, the number of pups per dam was adjusted to 5 and the dams were randomly allocated into five groups of 5 dams each. Dams in groups I, II, III, IV and V were administered distilled water (DW: 2 ml/kg) and metochlopromide (MET: 15 mg/kg), resveratrol (RES: 5: mg/kg), n-hexane leaf extract of *L. taraxacifolia* (LTF: 250 mg/kg) and their combination (CO; RES + LTF: 5+250 mg/kg) for 12 days (Cai *et al*., 2015). Serum was harvested and assayed with rat specific Prl and Oxt ELISA kits. The concentration of Prl was highest ($P < 0.05$) in LTF group when compared to other groups. Paradoxically, CO had lower ($P > 0.05$) concentration of Prl when compared to LTF and RES groups. However, Oxt concentration was highest ($P < 0.05$) in CO group when compared to other groups. The LTF group had higher ($P < 0.05$) Oxt concentration than MET and RES groups. In conclusion, *L. taraxacifolia* stimulated hyper prolactinaemia, while its combination with resveratrol caused increased serum oxytocin levels. Therefore, it is recommended that LTF could be beneficial as a galactogogue, while its coadministration with RES could serve as a potent stimulator of the milk let down reflex.

EFFECTS OF SOYA BEAN SUPPLEMENTS ON BLOOD GLUCOSE LEVELS AND LIPID PROFILE OF ALLOXAN INDUCED DIABETES MELLITUS IN WISTAR RATS

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Uncontrolled diabetes leads to several complications that affect many organs of the body. The health- beneficial effects of dietary fibres and antioxidants derived from plant food sources including soya beans are being extensively studied. This study aimed to evaluate the effects of soya bean supplements on blood glucose levels and lipid profile of alloxan-induced diabetes mellitus in Wistar rats. Twenty albino Wistar rats of both sexes between 120-150 grams were used for this study. Diabetes mellitus was induced by a single intraperitoneal injection of alloxan monohydrate at a dose of 150mg/kg body weight. Rats having fasting blood glucose levels of 200mg/dL and above after the induction were used for the study. The diabetic rats were grouped into four groups of five rats each: Group 1 (negative control) received distilled water orally for two weeks; Group 2 (positive control) were administered 5mg/kg body weight of glibenclamide orally for two weeks; Groups 3 and 4 were fed with 25% and 50% soya bean supplements respectively for two weeks. The fasting blood glucose levels were determined at intervals of 0, 1, 3, 6, 9 and 12 days respectively using a digital glucometer based on the glucose oxidase method. The animals were anaesthetised at the time of sacrifice by being placed in a sealed inhalation jar containing chloroform-soaked cotton wool. Blood samples were taken from all the groups for the determination of lipid profile. Data obtained were analysed using analysis of variance (ANOVA). The fasting blood glucose levels and lipid profile were significantly reduced ($P \leq 0.05$) in the soya beans supplemented group as compared with the control group after the two weeks of supplementation. **S**oya bean supplementation was found to have blood-glucose lowering potential and anti-lipidaemic activity in Alloxan-induced diabetic Wistar rats.

MODULATORY ROLE OF TAURINE IN ALLOXAN-INDUCED OXIDATIVE STRESS, BODY WEIGHT AND BLOOD GLUCOSE CHANGES IN WISTAR RATS

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In this study, the preventive effect of taurine on body weight, blood glucose level, oxidative stress and lipid peroxidation in alloxan-induced diabetes mellitus in wistar rats was determined. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Blood glucose level was measured after 72 hours of induction and diabetes was considered in animals with blood glucose level greater than 200 mg/Dl with Accu-Check Active Glucometer. The rats were grouped into (5) groups of five animals each n = (5) Group 1: Normal control, Group 2: diabetes untreated, Group 3: diabetes treated with 100 mg/kg taurine, Group 4: diabetes treated with 200 mg/kg taurine, and Group 5: diabetes treated with glibenclamide (1 mg/ml). Blood glucose and body weight of the rats were measured on weekly basis for three consecutive weeks. At the end of the treatment, all animals were sacrificed and blood samples were collected for serum extraction and determination of oxidative stress biomarkers and lipid peroxidation. The result of the present study shows a significant ($P < 0.05$) decrease in body weight and blood glucose level in groups that received 100 and 200 mg/ kg of taurine compared with diabetes untreated group alone at 21 days of the experiment. For oxidative stress biomarkers superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), P values < 0.05 were considered significant. Lipid peroxidation activities also showed a significant ($P < 0.05$) decrease in serum malondialdehyde (MDA) concentrations in the treatment groups. In conclusion, this study revealed that taurine could be a preventive supplement against oxidative stress in alloxan-induced diabetes and could be a potent supplement to mitigate against its occurrence.

EFFECT OF SELENIUM YEAST SUPPLEMENTATION ON SOME OXIDATIVE STRESS BIOMAKERS IN STZ-INDUCED DIABETIC NEUROPATHY IN WISTAR RATS

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Diabetic neuropathy is a long-term complication of diabetes, which affects half of the diabetic population. There is no prophylactic therapy against painful diabetic neuropathy. Current approaches are restricted to alleviating established pain by using drugs that are effective in other pain conditions and hoping to find one with enough efficacy and minimal side effect to be useful. The study investigated the effect of selenium yeast supplementation, an antioxidant and anti-inflammatory compound on some oxidative stress biomarkers in streptozotocin-induced diabetic neuropathy in Wistar rats. Thirty (30) adult Wistar rats with an average weight of 150 g were randomly divided into groups of five rats each (n=5). Diabetic neuropathy was induced by single intraperitoneal injection of 60 mg/kg streptozotocin dissolved in 0.1ml citrate buffer (pH 4.5). Groups I, II and III received 1mg/kg distilled water and aspirin (300mg/kg) while groups IV and V received 0.2 mg/kg and 0.3 mg/kg selenium yeast respectively. Blood plasma glucose concentration was determined weekly for four weeks. At the end of the fourth week, rats were euthanized and blood samples were collected and used to estimate for Malondialdehyde, glutathione peroxidase and superoxide dismutase concentration. The result showed that Streptozotocin (STZ) significantly ($p < 0.05$) increased MDA concentration while SOD and GPx levels were significantly reduced. Selenium yeast supplementation significantly enhanced antioxidant enzyme activity ($p < 0.05$). The results demonstrated that selenium yeast supplementation was beneficial in diabetic neuropathy via attenuation of oxidative stress. This suggests that Selenium yeast (0.2mg/kg) has potentials for the prevention of diabetic-induced neuropathy.

INVESTIGATION OF EFFECTS OF HYDROMETHANOLIC LEAF EXTRACT OF *CNIDOSCOLUS ACONITIFOLIUS* ON SOME LIVER ENZYMES AND HISTOLOGY IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS.

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The potency of plants are largely due to their phytochemicals constituents. These phytochemicals often times affects biochemical activities in animals and humans, thus the reason for the attention due to possible adverse effects or benefits, especially on liver enzymes and histology. This study is aimed at investigating the effects of hydromethanolic leaf extracts of *Cnidoscolus aconitifolius* on liver enzyme and histology in streptozotocin induced diabetic wistar rats. Thirty wistar rats with an average weight of 230grams were randomly assigned into five groups of six animals each. Group 1, served as negative control (non-diabetic) and received normal chow and water *ad libitum*, group 2 served as positive control group and received 10mg/kg bw of glibenclamide, groups 3, 4 and 5 served experimental group and received 100mg/kg bw, 150mg/kg bw and 200mg/kg body weight induced of C.A orally for 28days after being induced with diabetes using streptozotocin. Phytochemical screening of the extract revealed the presence of highly abundant level of alkaloids and flavonoids with moderate levels of tannins, phlobotannins, saponins, terpenes cardiac glycoside and cynogenetic glycoside. Administration of the extract shows significant ($p< 0.05$) increase in AST, ALT, ALP concentrations and alteration of the hepatic cells in the experimental group. The biological active phytochemicals in the hydromethanolic leaf extract of C.A may disrupt the liver enzymes, thus inducing cell rupture in the hepatocellular architecture, and might be hepatotoxic.

EFFECT OF AQUEOUS CRUDE EXTRACT OF *SENNA OCCIDENTALIS* LEAVES ON ANGIOTENSIN CONVERTING ENZYME (ACE) ACTIVITY IN HIGH-FAT-DIET INDUCED OBESITY IN WISTAR RATS

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Hypertension is one of the most common diseases across the globe responsible for many deaths resulting from some of its serious complications. There is a constant quest for better and safer medications in order to improve patients' quality of life. *Senna occidentalis* is extensively used in folklore medicine to treat several illnesses with little validation of its therapeutic benefits. Thus, the scientific evaluation of the effect of aqueous extract of *Senna occidentalis* on angiotensin converting enzyme (ACE) in obese Wistar rats was carried out in the present study. Samples from kidney, lung and heart tissues from 30 animals were used for the study which lasted for 3 weeks. Experimental animals were grouped into six (n=5) as normal control, obese control, Captopril 1.07mg/kgbw/day, obese + 100 mg/kgbw/day extract and obese + 250 mg/kgbw/day extract groups. Obesity was induced by feeding the animals with high-fat diet for eight weeks after which treatment was embarked upon. From the result of the present study, ACE activity was found to increase significantly (P<0.05) in lung, kidney and heart in obese control rats when compared to those of normal control group. ACE activity in kidney, lung and heart of obese Wistar rats treated with aqueous crude extract of *Senna occidentalis* at doses of 100mg/kg bw/day and 250mg/kg bw/day was found to decrease significantly (P<0.05) at the dose of 250mg/kg bw/day when compared to obese untreated group. ACE activity of normal group treated with 250mg/kg bw/day was found to decrease but not significantly (P<0.05) when compared to both normal control and obese untreated groups. The observed effect in the present study could attributed to active principles present in the extract. Thus, aqueous extract of *Senna occidentalis* can be relevant in the management of hypertension.

EFFECT OF L-CITRULLINE ON HYPERLIPIDEMIC WISTAR RATS

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Hyperlipidemia is a common predicament in society due to change of lifestyle and food practice.The study was designed to evaluate the effect of l-citrulline on hyperlipidemia. Twenty five male Wistar rats were divided into five groups of 5 animals each. (n=5) and treated for 21 days every 48hr interval. Group I (Normal Control), Groups II (Hyperlipidemic control), Group III (P-407+ Atorvastatin), Group IV (P-407+ 400mg l-citrulline), Group V (P-407+ 800mg l-citrulline). At the end of the experiment, blood samples were collected and the serum separated for evaluation of lipid profiles. L-Citrulline decreases total cholesterol (796.84±42.962 mg/dl) vs (732.21±13.77 mg/dl, 721.63±9.293 mg/dl) but however, was not significant (P < 0.05). Triglyceride level was significantly (p<0.05) decreased in a dose dependent manner (319.60 ± 11.58) vs (147.60±15.52, 139.40± 15.13) However, was not significant (P < 0.05). High density lipoprotein was increased (274.75±20.4) vs (319.30±27.2, 320.80±7.94) but however, was not significant (P < 0.05). Low density lipoprotein cholesterol decreased significantly (p<0.05) in a dose dependent manner (333.53 ± 30.24) vs (234.73±31.12, 175.30±8.21). In conclusion, L-citrulline decreases lipid profile in hyperlipidemia induced male wistar rats after 21 days oral administration.

COMPARATIVE EVALUATION OF GALACTOPOIETIC EFFECTS OF HEXANE LEAF EXTRACT OF *LAUNAEA TARAXACIFOLIA* AND RESVERATROL IN LACTATING FEMALE WISTAR RATS

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The inadequacy of milk supply to meet the increasing human population coupled with a decreasing livestock population has necessitated the need for a potent galactogoue, particularly from plant origin. This study was aimed at evaluating the galactopoietic effects of n-hexane leaf extract of *L. taraxacifolia* (LTF), resveratrol and their combination in Wistar rats. Twenty-five mature nulliparous female Wistar rats were bred and following parturition, the number of pups per dam was adjusted to 5. The dams and pups were divided into five groups of 5 dams each. The dams in groups I, II, III, IV and V were treated by gavage daily at 7:00 pm with distilled water (DW, 2 ml/kg), metochlopramide (MET, 15 mg/kg), resveratrol (RES, 5 mg/kg), LTF (250 mg/kg) and the combination of RES and LTF (CO, 5 + 250 mg/kg), respectively. The administration commenced on day 2 through to day 14 of lactation. Pups were weighed three times daily at 13:00 (W_1), 17:00 (W_2) and 18:00 (W_3) hours, respectively. The W_1, $W_3 - W_2$ and $(W_1 - W_0)/W_0$ denote daily weight of pups, milk yield and percentage growth rate, respectively. The results showed that milk yield was non significantly (P > 0.05) higher in CO and LTF than the yield obtained in RES, MET and DW groups. The daily weight gain of pups was significantly greater (P < 0.05) in LTF, RES and CO groups when compared to DW group. Although the LTF group had higher daily weight gain than the RES and CO groups, it was not significant (P > 0.05). However, percentage growth rate was significantly lower in MET and LTF groups compared to other groups; while RES had significantly higher percentage growth rate than CO. In conclusion, *Launaea taraxacifolia* and resveratrol exhibited galactopoietic potentials individually, and synergistically when co-administered.

IMPAIRMENT OF CARDIOVASCULAR FUNCTION INDICES IN MALE WISTAR RATS INDUCED BY ALUMINIUM-TAINTED WATER: ATHEROGENIC INDICES AND PREDICTOR RATIO ASSESSMENT

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Cardiovascular disease (CVD) epidemic is an ever-growing health problem and remains the leading cause of death in all regions of the world. Currently, there is growing evidence that exposure to metal pollutant is a risk factor that disturbs lipid metabolism with increase risk for CVD. The present work aimed to evaluate the toxicity of aluminium chloride - tainted drinking water ($AlCl_3$) on lipid profile in male wistar rats in assessing cardiovascular risk by using atherogenic indices and prediction ratio. Fifty (50) male Wistar rats were randomly assigned to five groups of 10 rats each. Group A was given normal drinking water whilst $AlCl_3$ treated groups B, C, D and E received 200, 400, 600 and 800mg/kg of $AlCl_3$ respectively via orogastric route once daily for 28 days. Thereafter, blood samples were collected for lipid profile analysis. Atherogenic indices like Castelli's Risk Index (CRI), Atherogenic Index of Plasma (AIP), and Atherogenic Coefficient (AC), lipid ratios and predictor ratios were determined. With values significantly different at $P \leq 0.05$, the estimated atherogenic indices- CR1-11, AC and CRI-1, ratios TG/HDL-c, TC-HDL-c/HDL-c, TC/HDL-c and low HDL-c/LDL-c, dose-dependently rose approximately and significantly differently determined cardiovascular risk in rats by $AlCl_3$. Predictor ratio however, revealed that AIP did not significantly impact the risk of cardiovascular disease in rats. In conclusion, exposure to $AlCl_3$ elicited concurrently dose-response proliferation of both dyslipidaemia and atherogenic indices differentially with resultant deleterious effect in cardiovascular cells and tissues in rats. AIP may not be an independent factor in $AlCl_3$ impacting the risk of CVD in rats. These might be the various possible mechanisms of aluminium toxicity in male rat cardiovascular risk.

CLOTTING TIME AND CALCIUM HOMEOSTASIS AMONGST POSTPARTUM HEMORRHAGE PATIENTS ATTENDING MURTALA MUHAMMED SPECIALIST HOSPITAL, KANO

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According to the World Health Organization, 15 women dies every hour as a result of obstetrics hemorrhage, with large proportion of these deaths occurring in developing countries. Several factors have been outlined as possible causes of postpartum hemorrhage (PPH) among which includes uterine atony. Serum calcium has been known to play a central role in vascular spasm, clotting mechanism and uterine tonicity, both of which affects uterine bleeding; hence disrupted calcium homeostasis may perhaps contribute to the incidence of PPH. Few local data is available on the relationship between PPH and clotting time, serum calcium and serum proteins. This study therefore assessed clotting time, serum calcium and phosphate levels as well as serum proteins among PPH patients and their normal delivery cohorts in Murtala Muhammad Specialist Hospital (MMSH), Kano, using a cross sectional comparative study design. Thirty (30) post partum hemorrhage patients matched with 30 cohorts of normal delivery were recruited after signing an informed consent. Blood samples were collected from the ante-cubital vein and transferred to a plain sample container for the estimation of serum analytes while clotting time was estimated using Dukes method. Sample analysis were done using specific kits obtained from Randox laboratories LTD, UK, all, according to the manufacturer's instructions. Data analysis was done using SPSS (V.20.0) and was summarized using mean±SD. An independent samples t-test was used to compare variables between the groups and statistical significance was set at 5% margin of error. The results indicated that PPH patients had significantly ($p=0.001$) higher clotting time, lower serum calcium, lower albumin and total serum protein than the controls. Serum phosphate was however not statistically different between the groups. In conclusion, the findings may point to an abnormality in calcium homeostasis of the PPH patients.

ASSESMENT OF MODULATORY ROLE OF CLOVE SUPPLEMENT ON LEAD INDUCED MEMORY IMPAIREMENT IN WISTAR RATS USING ELEVATED T AND Y MAZES.

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Cloves are dried flower buds of *syzygium aromaticum*, a tree of the myrtle family, which is evergreen tropical plant that flowers twice every year. It's an important medicinal plant due to it's wide range of pharmacological effects. Lead intoxication affects many systems of the body including the cardiovascular, renal, and reproductive systems. Its most detrimental effects occur in the nervous system, where it blocks N-Methyl-D-aspartate receptors. This study assessed the modulatory effect of clove supplement on lead induced memory impairment in Wistar rats. Twenty wistar rats (100-150g) were randomly divided into four groups (IIV) of five rats each. Group I served as control. Group II was treated with 0.1mg/kg of distilled water after pre-treatment with 10mg/kg of lead acetate. Grouped III was treated with clove supplement, 3% of clove supplement was mixed with the animal feed and 10mg/kg of lead acetate was administered. Group IV was also treated with clove supplement, where 6% was mixed with the animal feed and 10mg/kg of lead acetate was administered. Lead treatments were via oral gavage for 14 days. Neurobehavioural paradigms of Y and elevated plus mazes were employed to assess the spatial learning and memory. Findings revealed that, lead induced memory impairment was reduced at 6% and 3% respectively. It can be concluded from these results that lead induced memory impairment can be modulated using clove supplement.

EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF L-CITRULLINE IN MICE AND ITS ANTISPASMODIC EFFECT ON ISOLATED RABBIT JEJUNUM

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L-citrulline is a non-essential amino acid that plays a vital role in the metabolism and regulation of nitric oxide. It is majorly synthesized in the small intestine and considered safe for consumption. However, there is paucity of literature on its anti-diarrhoeal and antispasmodic effects. Hence, these study investigates the anti-diarrhoeal activity of L-citrulline in mice and its antispasmodic effect on isolated rabbit jejunum. Castor oil induced diarrhoea model was used for the antidiarrhoeal studies. Test groups received L-citrulline 300 and 600 mg/kg respectively, positive control group received Loperamide 5 mg/kg while negative control group received normal saline 2 ml/kg. All administrations were via oral route. The antispasmodic effect was evaluated using isolated tissue experiment. L-citrulline 300 and 600 mg/kg caused a reduction in the mean number of wet feaces when compared to the normal saline treated group. Diarrhoeal protections of 93.33% and 55.49% were observed at 300 and 600 mg/kg of L-citrulline, respectively. L-citrulline showed more anti-diarrheal effect at the lower dose of 300mg/kg and the result obtained was similar to that of Loperamide which showed higher percent protection. L-citrulline in the antispasmodic studies showed a concentration dependent decrease in strength of contractions of the rabbit jejunum which was similar to what was observed on administration of Adrenaline. When L-citrulline was interacted with Acetylcholine, there was a blockade of effect similar to that observed on interaction of the same concentration of Acetylcholine with Atropine. This indicates that L-citrulline might have inhibited the contractions of the rabbit jejunum via stimulating adrenoceptors and/or blocking cholinergic receptors. Therefore, from the results obtained in this study it may be suggested that L-citrulline possesses some antidiarrhoeal potentials.

SOME HEMATOLOGICAL INDICES AND LIVER FUNCTION PARAMETERS AMONG WORKERS EXPOSED TO CHRONIC INHALATION OF LIQUEFIED PETROLEUM GAS (COOKING GAS) IN CALABAR, NIGERIA

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Gas workers in Calabar, Cross River State are chronically exposed to Liquefied Petroleum Gas (LPG) or cooking gas. This gases being lipophilic in nature, after inhalation, are absorbed into the blood, and enters into the tissues of their body. This study investigated some hematologic and liver function parameters among workers exposed to chronic inhalation of LPG in Calabar. A total of ninety (90) male subjects consisting of 45 control and 45 LPG Workers were used for this study. FBC, liver enzymes, plasma proteins and bilirubin concentration were assessed. Air quality studies were carried out and questionnaires were filled. The results showed a significant increase in RBC, PCV, Hb conc. MCV, MCHC and MCHC of the test group when compared to the control ($P< 0.001$). A significant increase in the total WBC, Lymphocyte and ESR of the test group was found when compared to the control group ($P<0.05$).A significant increase in total bilirubin, Conjugated bilirubin ALP, and globulin ratio of the test group was observed when compared to the control ($P<0.001$), while a significant decrease in total protein and albumin ratio was found in the test group compared to the control group ($P<0.001$). Other health related complaints such as Fatigue, itches, headache, rashes, abdominal pains, jaundice and dizziness were also more common in the LPG exposed workers than the controls. In conclusion, chronic inhalation of LPG without necessary precautions causes several changes on hematological indices and liver function parameters of gas workers and could lead to blood diseases and liver malfunction.

BIOSIGNATURE OF TOLUENE DIISOCYANATE-INDUCED ASTHMA IN FEMALE BALB/C MICE: THE ROLE OF β-CATENIN MEDIATED AIRWAY EPITHELIAL INTEGRITY

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Highly reactive low molecular weight compound, toluene diisocyanate (TDI), is a common inducer of work-related asthma especially in industrialised nations. Oxidative stress, airway dysfunction and inflammation have been related to chemical-induced occupational asthma. The role of adherence junction proteins in the maintenance of airway integrity was previously reported. This study evaluated in-depth pathophysiology of TDI-induced airway epithelial desquamation as it relates to T-helper cell endotype. Female Balb/c mice were dermally sensitised and intranasally exposed to TDI following a 21-day exposure regimen. After the final exposure, bronchoalveolar lavage fluid (BALF), lung samples and serum were collected. Analysis of BALF leukocytes showed a significant rise in both total and differential leukocyte counts, with predominant airway neutrophilia. Similarly, BALF reactive oxygen species were significantly increased by TDI exposure. Lung tissue inflammatory cells infiltrates, mucus production and collagen deposition were similarly higher among TDI-exposed animals. In addition to epithelial desquamation, aberrant distribution of E-cadherin and β-catenin complexes were observed upon TDI exposure. A significant increase in Th2 cytokines (IL-4, IL-5 and IL-13) coupled with rise in IFN-γ, IgE and IgG inferred Th1/Th2 asthma endotype. Phenotypically, airway resistance and dynamic compliance were significantly altered by TDI-exposure. Whereas, the expression of upstream and downstream oxidative and inflammatory

mediators such as Akt, p38, Nrf2 activation and HO-1 were similarly affected by TDI exposure. These findings have delineated molecular targets for the management of chemical-induced asthma.

EVALUATION OF AGE- AND SEX-RELATED EFFECTS OF RAMADAN FASTING ON BODY WEIGHT OF APPARENTLY HEALTHY PERSONS IN KANO METROPOLIS, NIGERIA

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Fasting in the Month of Ramadan is physiologically a robust non-genetic, as well as non-pharmacological observance that is defined as a complete abstention from eating any solid foods or liquids every day between the months of May and June every year, or as the case may be depending on Geographical location. The purpose of the study was to investigate age- and sex-related variability in weight and BMI before and after Ramadan Fasting. Ages, weights and heights of all subjects were taken and BMI calculated using the *Adolphe Quetelet's standard formula (Kg/m2).* Paired sampling and independent sample t-test were used to determine changes in ages and sexual dimorphism, respectively. There were significant increases in body weight and BMI in male participants after fasting observances. Based on age group, the increase in weight and BMI was only significant among male participants within middle-aged. Gender wise comparison of weight and BMI revealed significant sexual dimorphism in weight only after Ramadan fasting, with higher mean value in males compared to females. However, sexual dimorphism based on age group showed significant differences in BMI among the middle age group, before the Ramadan Fasting, with higher mean value in females. It was concluded that, Ramadan Fasting has positive effects on body weight and BMI in middle-aged males; sexual dimorphism in body weight and BMI affects only the middle-age group.

EVALUATION OF POSSIBLE ANTI-DEPRESSANT EFFECT OF ALPHA-LIPOIC ACID ON CHRONIC MILD STRESS MOUSE MODEL OF DEPRESSION

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World Health Organization (WHO) reported that depression is the 4th highest contributor to the global burden of disease and is predicted to be the first by 2030. Alpha-Lipoic Acid (ALA) is a potential antioxidant; synthesized in the mitochondria, it has the potential to increase insulin sensitivity as one of its wide range of benefits. Increase in insulin sensitivity is found to elevate serotonin level via increasing its precursor tryptophan. This research was aimed to evaluate effects of ALA on chronic mild stress model of depression. Fifteen mice of both sexes were used in this study. They were grouped into three groups of five mice each. Group 1 received 10 ml/kg Normal Saline (NS), Group 2 received ALA 200 mg/kg and Group 3 received Flouxetine 20 mg/kg. At the end of two weeks application of Chronic Mild Stressors (CMS), treatment was administered for another two weeks. Tail Suspension Test (TST) was conducted before and after application of CMS and treatments. Result of TST after treatment revealed a statistically significant difference between ALA and NS in immobility time (behavioral despair). In Open Field Test (OFT), no statistically significant difference was observed between NS and treatment groups in line crossing (locomotor activity). In Novel Object Recognition Test (NORT), no significant difference was observed in percentage preference between NS and ALA but there was statistically significant difference between Flouxetine and NS. In conclusion, ALA 200 mg/kg has shown a potential anti-depressant like effect in TST by decreasing the immobility time of mice subjected to CMS but has no effect on locomotor activity (line crossing) and cognitive function as seen in OFT and NORT respectively.

EFFECTS OF PHOSPHODIESTERASE 5 ENZYME INHIBITORS ON COGNITION IN MALE ADULT WISTAR RATS

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Neurodegenerative diseases are said to be associated with dementia and treatment has not been fully successful. Phosphodiesterases (PDEs) are class of enzymes that reverse the formation of second messenger cyclic AMP and cyclic GMP thereby terminating the physiological response of their first messengers. PDEs inhibitors such as sildenafil and tadalafil are hypothesized to prolong the physiological action of glutamate thereby believed to partake in long term potentiating (LTP). In this study we explore the possibility of using these drugs to enhance memory. Twenty four 24 male wistar rats (150g) were divided into four groups of six each (n=6). Group I, II, III and IV received 10 ml/kg of distilled water (Negative control), 5 mg/kg of sildenafil, 5 mg/kg of tadalafil and 20 mg/kg of piracetam (positive control) respectively. All administrations were done via oral gavage for two weeks. Y maze and NORT paradigms were used to assay cognitive functions. The result of our finding showed a statistically significant decrease on short term memory in the sildenafil and tadalafil treated rats groups when compared with the control groups. Using Nobel Object Recognition Task (NORT), sildenafil and tadalafil treated groups were found have significantly increased preference core indicating memory enhancement when compared with the negative control group. The piracetam treated group which is a

standard memory enhancer showed statistically significant increase in the preference score compare to all other groups. In conclusion the study illustrates that sildenafil and tadalafil posses potentials for memory enhancement in rats treated groups. The study also indicated that tadalafil has more potent memory enhancement capability on the rats treated group.

ELECTROLYTE AND OXIDATIVE STRESS PROFILE OF HEALTHY ADULT POPULATION IN ZARIA, NIGERIA AND THEIR RELATIONSHIP WITH EXPERIMENTAL PAIN RESPONSE

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Oxidative stress (OS) results from accumulation of reactive species, beyond the endogenous anti-oxidant capacity. OS is known to impair physiological functions, which can alter health and wellbeing. This reactive species are detoxified by endogenous anti-oxidant enzymes, which help to keep the system in a state of homeostasis. Electrolytes are known to serve crucial functions in the body that include regulation of water balance, maintaining pH, and transport of substances. Electrolyte imbalance can be a marker of many disorders. This study investigated the electrolyte and oxidative stress profile of a healthy adult population in Zaria, Nigeria and their relationship with experimental pain outcome. Participants were apparently healthy adult volunteers between the ages of 20 to 65 years, and drawn from the city of Zaria and its environs. Experimental pain was induced using pressure algometry. About 5 ml of blood was collected for determination of serum electrolytes, MDA, GSH and SOD. The results showed that serum concentrations of sodium, potassium and chloride as well as oxidative stress profile did not vary with sex, age and ethnicity among the study population. There was a significant negative correlation between pressure pain threshold and serum concentration of potassium ($r = 0.2330$, $p = 0.003$) and chloride ($r = 0.2126$, $p = 0.007$), while serum sodium correlated positively ($r = 0.3439$, $p = 0.000$). Serum MDA, SOD and GSH did not show statistically significant correlation with pressure pain threshold ($p > 0.05$). In conclusion, serum electrolytes correlate significantly with experimental pressure pain threshold among healthy adult population in Zaria, Nigeria.

LEVELS OF CYTOKINES IN RELATION TO MATERNAL GROUP B *STREPTOCOCCUS* COLONIZATION AT DELIVERY

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Infection with Group B *Streptococcus* (GBS) causes high morbidity and mortality among newborn babies. Maternal GBS colonization during pregnancy is a leading risk factor for neonatal GBS disease. Apart from the culture and the polymerase chain reaction (PCR) methods recommended by Centers for Disease Control and Prevention, studies have shown that cytokines produced by immune cells in response to the presence of bacteria may also be useful at detecting bacterial infections. We therefore investigated whether maternal serum interleukin (IL)-6, IL-8 and IL-10 are useful indicators to predict GBS colonization at delivery. Healthy HIV negative pregnant women, free of any medical condition and not receiving antibiotics, were recruited for the study (n = 136). Vaginal swabs and venous blood were collected at the time of delivery. Swabs were used to isolate GBS using two methods: culture method and quantitative PCR. A mother was deemed colonized by GBS if either the PCR or the culture were positive. Maternal serum was used for cytokine analysis using high sensitivity premixed magnetic Luminex performance assays (R&D Systems). Cytokines values were normalized using a logarithm transformation. Of the 136 Participants, 47 (35%) were colonized with GBS. At delivery, there was no statistically significant difference in logged concentrations of IL-6 (P=0.8), IL-8 (P=0.5) or IL-10 (P=0.9) according to colonization status. Our results show that maternal serum IL-6, IL-8 and IL-10 are not reliable markers to predict GBS colonization at delivery.

IN VITRO AND *IN VIVO* ANTIDIABETIC PROPERTY OF MORINGA OLIEFERA CYCLOTIDE FRACTION

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Plant cyclotides are chemically stable and beneficial for medicinal application if they possess such properties. *Moringa Oliefera* exhibits anti-diabetic activity; however whether its cyclotide fraction also exhibits anti-diabetic effects is unknown. The present study was thus designed to determine the *in vitro* and *in vivo* anti-diabetic effect of *Moringa Oliefera* cyclotides (MOC). MOC fraction was obtained by solvent extraction and peptide purification. Toxicity assessment was carried out using the brine

shrimps lethality test (BST). *In vitro* antidiabetic property of MOC was assessed by α-amylase inhibitory activity using the dinitrosalicylic acid method. *In vivo* antidiabetic property of MOC was assessed in a type 2 diabetic (T2D) guinea pig model. BST of MOC was non-toxic (LC_{50}; 37561.3 µg/ml). MOC exhibited maximum α-amylase inhibitory activity of 88.3 % at 400 µg/ml. T2D animals presented elevated fasting blood glucose (FBG) (134.3±13.7 vs 89±9.8 mg/dl), reduced glucose utilization, normal insulin levels but reduced hepatic insulin receptor substrate-1 (IRS-1) (3.4±0.7 vs 11.1±1.7 %) and skeletal muscle Glut 4 expression (21.8±4.4 vs 30.5±5.1 %) when compared with control. Treatment of T2D animals with glibenclimide and MOC (10 mg/kg) reduced FBG to 108±11.3 and 105±13.1 mg/dl respectively, increased glucose utilization; increased expressions of hepatic IRS-1 and skeletal muscle Glut 4 ($P< 0.05$) respectively. While MOC treatment did not significantly alter plasma insulin levels in diabetic animals, glibenclimide significantly increased plasma insulin levels (0.68±0.04 vs 0.25±0.03 ng/ml). In conclusion MOC is nontoxic. MOC possesses anti-diabetic activity, probably mediated via inhibition of α-amylase activity and improvement of insulin action.

PINEAL AND HYPOPHYSEAL RESPONSES TO SELENIUM TREATMENTS IN LIGHT DEPRIVED ADULT FEMALE WISTAR RATS

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Dark is a generally known potent stimulus for melatonin secretion. Studies have also reported that pineal and hypophyseal endocrine secretions are influenced by nutritional factors. The study is designed to investigate the combined effect of selenium supplementations and light deprivation on pineal and hypophyseal secretions in female wistar rats. 36 female cyclical wistar rats were divided into vehicle, high selenium (HS), low selenium (LS), light deprived (LD), LD+HS and LD+LS. Rats were orally administered 150µg/kg and 100µg/kg of sodium selenite for two weeks. While light deprived rats were maintained under 6hr light/18hr dark cycle, other rats were under natural 12hr light/12hr dark cycle. The result showed that light deprivation led to significant decrease ($P<0.05$) in follicle stimulating hormone (FSH) secretion and a significant increase ($P<0.05$) in plasma melatonin. Selenium supplementations at both doses also improved LH secretion but had no effect on plasma prolactin. At high dose of selenium supplementation, there was an increase in melatonin secretion. In light deprived rats, selenium supplementations caused a significant decrease ($P<0.05$) in melatonin secretion at both doses but there was no significant change in plasma levels of FSH, LH and prolactin. The findings indicate differential responses of pineal and hypophyseal glands to light deprivation and selenium treatments.

AMELIORATIVE POTENTIAL OF *ALLIUM CEPA* ON P53 AND BCL2 EXPRESSION, DNA FRAGMENTATION IN LIVER OF CADMIUM SULPHATE TREATED RATS.

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Cadmium (Cd), one of the highly toxic metals to both humans and animals has been known to be cytotoxic and responsible for inducing free radical-dependent DNA damage in cells. *Allium cepa* is a known antioxidant with ameliorative potentials on cadmium-induced organ toxicity. Therefore this study was carried out to evaluate the effects of *Allium cepa* extract on DNA fragmentation, p53 and Bcl2 expression in liver of cadmium sulphate treated rats. Twenty adult male wistar rats weighing 160-180g were used for this study. They were divided into four groups, group one served as control, group two (Cd group) were treated with 15mg/kg $CdSO_4$, Group three (Cd + AcE group) were treated simultaneously with 15mg/kg $CdSO_4$ and *Allium cepa Extract* (1mL/100g BW) while the fourth group (AcE group) received *Allium cepa* extract (1mL/100g BW) alone. All the treatments were given orally for 28 days. Liver Superoxide Dismutase (SOD), Catalase (CAT) activities and % DNA fragmentation were examined spectrophotometrically while immunohistochemical expression of Tumor Suppressor protein (p53) and cytoplasmic Bcl2 were also studied. Exposure to cadmium significantly decreased SOD and CAT activities. It significantly increased % DNA fragmentation and Bcl2 expression and inhibited p53 expression. Decreased SOD and CAT activities, increased DNA fragmentation and Bcl2 expression together with inhibition of p53 expression by cadmium were all ameliorated with *Allium cepa* treatments. In conclusion, *Allium cepa* inhibits liver DNA damage by increasing antioxidant activities and inhibiting Bcl2 expression while promoting p53 expression.

AMELIORATIVE EFFECTS OF MANGANESE GLYCINATE AGAINST INCREASED LIVER ENZYMES IN RATS EXPOSED TO WATER IMMERSION RESTRAINT STRESS

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Stress response is crucial, as it enhances the organism's chances for survival. It may become overwhelmed by severe stress, resulting in organ injury, manifesting as diseases like gastric ulcers. Water-immersion restraint stress (WIRS) is known to cause increased levels of various serum enzymes in rats. The present study examined the effects of manganese glycinate on serum levels of the liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in rats exposed to WIRS. Fifteen (15) male Wistar rats (150-170 g) were divided into 3 groups of 5 rats each: (I) passive control; (II) active control; and (III) pre-treated with manganese glycinate and subjected to WIRS for 3.5 hours. At the end of the experiment, blood samples were collected through decapitation for haematological and biochemical analyses. The result showed that acute WIRS significantly ($p<0.05$)

increased plasma ALT, AST and ALP activities in the active control group, when compared with the passive control group. Pre-treatment of rats with manganese glycinate showed significant amelioration of these changes induced by WIRS. It was concluded that manganese glycinate pre-treatment has possible anti-oxidant and protective effects against liver damage as may be caused by WIRS in rats.

ANTIDEPRESSANT STUDIES OF METHANOL LEAF EXTRACT OF *Ziziphuss Mauritiana* IN ALBINO SWISS MICE

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The leaves of *Ziziphus mauritiana* have been reported to be used in the management of depressive illnesses in traditional medicine. The antidepressant activity of the methanol leaf extract of the plant was evaluated using tail suspension test and forced swim test at the doses of 25 mg/kg, 50 mg/kg, 100 mg/kg, and 200 mg/kg, the effect of the extract on recognition memory, motor coordination, and exploration behaviour was evaluated using novel object recognition test, beam walking assay test and open field test respectively. Data was and represented as± SEM and analyzed using one-way analysis of variance (ANOVA), followed by the Bonferroni *post hoc* test where P< 0.05 was considered significant. The result showed a statistical significant decrease in duration of immobility $p < 0.001$ on TST, when compared with the control. Imipramine (20 mg/kg) was used as standard antidepressant drug. It may be concluded that the methanol leaf extract of *Ziziphus mauritiana* possesses antidepressant property.

INFLUENCE OF AGE, SEX AND EDUCATIONAL STATUS ON SELF-MEDICATION AMONG FARMERS AND ITS ASSOCIATION WITH PEPTIC ULCER DISEASE

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The trend towards self-medication is at an increasingly alarming rate in our communities and it is becoming easier to procure more drugs over-the-counter (OTC) now than ever before. Peptic ulcer disease (PUD) incidence due to *Helicobacter pylori* infection is decreasing, however most cases now can be attributed to other secondary factors such as ingestion of Non-Steroidal Anti-inflammatory Drugs (NSAIDs). The readily availability of NSAIDS encourages the farmers to self-medicate. This study aims to assess the influence of age, sex and educational status on self-medication of NSAIDs among farmers in Shika District of Sabon Gari Local Government Area of Kaduna State. This was a descriptive cross-sectional study. A total of 220 questionnaires were administered. Study participants were sampled using a systematic random sampling technique and an interviewer administered structured questionnaire was used to collect data which was entered and analyzed using SPSS v17. About 91.8% of the farmers were found to self-medicate. Farmers ≤ 40 years were found to have about five times higher odds to self-medicate than farmers > 40 years of age (cOR=5.04; 95% CI 1.87- 13.58). Sex and educational status were not found to be statistically associated with self-medication. There is an alarmingly high prevalence of self-medication among young farmers. Younger farmers may have high risk of developing NSAID-induced peptic ulcer.

ASSESSMENT OF MILK YIELD AND SOME LACTOGENIC HORMONES IN FEMALE LACTATING WISTAR RATS FOLLOWING FERMENTED SOYA BEAN AND ASCORBIC ACID SUPPLEMENTATION

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Milk is essential for optimal feeding of infants and has a direct impact on growth, development, and health in neonatal period. This study was designed to assess milk yield following Soya bean and Ascorbic acid supplementation. At parturition, the animals were randomly divided into seven groups of five rats each (n=5) and treated as follows: Group I: (Normal control) was given normal feed and normal saline, orally (1 ml/kg bw), Group II: metoclopramide (5 mg/kg bw) ,Group III: 100 mg/kg bw of Vitamin C, Groups IV, V and VI were given soya bean supplement thus; 10%, 20% and 40%, respectively. Group VII was co-administered with 20% soya bean supplement and Vitamin C (100 mg/kg bw). Treatment was done for the period of ten (10) days at 06:00 hours daily and the animals were euthanized at the end of the experiment. Serum levels of prolactin, oxytocin and milk yield 18 and 23 hours after gavage were evaluated. The result on serum prolactin didn't show any statistical significant increase although there was increase in all the supplements treated groups compared to control except in the group administered 20% of Soya bean supplement. Serum prolactin level was highest in the Soya bean and Vitamin C co-administered group compared to control. Serum oxytocin level was statistically significant ($P< 0.05$) in the metoclopramide treated group (404.20±19.40 vs 342.80±20.30) compared to control. In this Soya bean supplement has been shown to increase serum prolactin and oxytocin level, resulting in a concomitant increase in milk yield

EVALUATION OF SOME LACTOGENIC HORMONES, MILK YIELD AND OXIDATIVE STRESS BIOMARKERS IN EXCLUSIVELY AND NON-EXCLUSIVELY BREASTFEEDING MOTHERS IN ZARIA

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The aim of the study was to evaluate some lactogenic hormones, milk yield and oxidative stress biomarkers in exclusively and non-exclusively breastfeeding mothers in Zaria. The population for this study consist of breastfeeding mothers attending postnatal care in Hayin Dogo primary health care centre Samaru - Zaria, aged 20 to 50yrs who practice different breastfeeding methods. A sample size of 60 was considered for the study. Five millilitre of venous blood was collected by vein puncture with sterile needle and the sera was used for hormonal and antioxidant assay. The result show a significant increase ($P< 0.05$) in milk index of the non-exclusive group when compared to the exclusive group; 0.11±0.012 (kg/child) vs 0.081±0.018 kg/child respectively. A significant increase ($P< 0.05$) in the serum prolactin level of the exclusive breastfeeding mothers was observed when compared to the non-exclusive breastfeeding mother 73.10±13.90ng/ml vs 45.67±14.33ng/ml respectively. Although there was an increase in the serum progesterone level of the non-exclusive breastfeeding mothers when compared to the exclusive breastfeeding mothers; 8.21±2.37ng/ml vs. 6.36±2.56ng/ml respectively, it was however not statistically significant. There was a non-significant increase ($P> 0.05$) in the serum estrogen level of the non-exclusive breastfeeding mothers (73.30±10.09pg/ml) when compared to the exclusive breastfeeding mothers (64.80±13.90pg/ml). Also, there were no statistical-significant increase ($P> 0.05$) in the serum MDA, SOD and GPx level of the exclusive breastfeeding mothers when compared to the non-exclusive breastfeeding mothers. It is therefore suggested that exclusively breastfeeding mothers should be well motivated and prepared to manage the stress associated with breastfeeding through up regulation of their antioxidant defence system.

COMPARATIVE STUDY OF THE ANTI-DIABETIC EFFECTS OF METHANOLIC EXTRACTS OF C*URCUMA LONGA* RHIZOMES AND S*PONDIAS MOMBIN* LEAVES IN ALLOXAN INDUCED DIABETES IN MALE WISTAR RATS

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Approximately eighty percent of deaths associated with diabetes mellitus the world over; occur in low and middle-income countries. Due to poverty, there is the tendency for patients to rely on orthodox medications with attendant adverse effects. Medicinal plants like *Curcuma longa* and *Spondias mombin* have been separately evaluated in ameliorating diabetic conditions. Therefore, this study attempts a comparative assessment of the anti-diabetic effects of methanolic extracts of C*urcuma longa* rhizomes and S*pondias mombin* leaves in alloxan-induced diabetic male wistar rats. Ninety rats were divided into nine groups of 10 animals each. Diabetes was induced using 200mg/kg/b.w of alloxan administered intraperitonially. The groups were treated as follows: 1: Non-diabetic control; 2: Untreated diabetic; 3: 200mg/kg/b.w *Spondias mombin;* 4: 400mg/kg/b.w *Spondias mombin;* 5: 500mg/kg/b.w C*urcuma longa*; 6: 1000mg/kg/b.w C*urcuma longa*; 7: 200mg/kg/b.w S*pondias mombin* + 500mg/kg/b.w Curcuma longa; 8: 400mg/kg/b.w S*pondias mombin* + 1000mg/kg/b.w C*urcuma longa*; 9: 0.6mg/kg/b.w. Glibenclamide. On day 43, blood was collected by cardiac puncture for determination of blood sugar and glycosylated haemoglobin concentration. A significant ($p< 0.05$) dose dependent decrease in the blood sugar and glycosylated haemoglobin levels were shown in all the treated groups compared to group 2. Furthermore, groups 7 and 8 showed a significant reduction ($p<0.05$) in blood sugar and glycosylated haemoglobin concentration compared to groups 3 to 6. S*pondias mombin* apparently showed better anti-diabetic effects compared to C*urcuma longa*. However, our result shows that combined treatment with S*pondias mombin* and C*urcuma longa* may potentiate the anti-diabetic effects seen in our experimental studies.

ACUTE AND SUB-ACUTE TOXICITY STUDIES ON METHANOLIC EXTRACT OF *Combretum dolichopetalum* LEAVES

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Combretum dolichopetalum leaves are used in African ethnomedicine as a blood booster, relief of menstrual pain, enhancement of labour, etc. However, to the best of our knowledge, systematic study regarding its toxicity profile has not been reported. The present study sought to carry out acute and sub-acute toxicity studies on *C. dolichopetalum* leaves. The studies were carried out on experimental mice and rats respectively using standard techniques. Results The LD50 of the extract was obtained as more than 5000 mg/kg body weight. Administration of graded doses (100, 200, 400 and 800 mg/kg) of the extract for 21 days resulted in increases in body weights, while blood cells (WBC), Neutrophils, red blood cells (RBC), packed cell volume (PCV), haemoglobin (HGB), mean corpuscular volume (MCV) and mean cell haemoglobin (MCH) of the rats; but did not affect ($P>0.05$) their monocytes, mean cell haemoglobin concentration (MCHC), platelet (PLT) levels. All doses of the extract did not affect ($P>0.05$) the sodium, potassium, chloride, bicarbonate, urea, creatinine, total and conjugated bilirubin, alanine and aspartate amino transaminase, aspartate amino transaminase, alkaline phosphatase activities; relative liver and kidney weights of the rats, a finding that was corroborated by histology of the liver and the kidney. The study provided a scientific rationale for the use of *Combretum dolichopetalum* leaves in Nigerian ethnomedicine as a blood booster. The study also revealed the safety in the usage of *C. dolichopetalum* leaves in Nigerian ethnomedicine

ADMINISTRATION OF FLAVONOIDS FROM *Hibiscus sabdariffa* TO SUCROSE-CONSUMING LACTATING RATS MAY PROGRAM INCREASED WEIGHT GAIN IN OFFSPRING LATER IN LIFE

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Incidence of metabolic dysfunction due to consumption of sugar sweetened beverage is on the increase (Miranda *et al*.,2005) and it is well established that its high intake by lactating dams is associated with development of metabolic dysfunction in offspring (Malik *et al*.,2010). *Hibiscus sabdariffa* (*HS)* with its phytochemicals, has shown prospects in prevention and treatment of obesity and metabolic dysfunction through unclear mechanisms (Alarcon-Aguilar *et al*.,2007). This study investigated the effect of administration of flavonoids from *HS* to sucrose consuming lactating rats on the offspring food intake and postnatal growth. 32 female Wistar rats were used for this study. They were divided into sucrose and non-sucrose groups and 8 sub-groups with 4 rats in each subgroup. Non-Sucrose groups were administered 10mg/kg, 20mg/kg and 50mg/kg of flavonoids from *HS*, the sucrose group were administered 30% sucrose with 10mg/kg, 20mg/kg and 50mg/kg of flavonoids from *HS* orally. Extract administration commenced on day 1 of lactation and ended on postnatal day 21. Blood samples were withdrawn from the offspring on postnatal day 42 for determination of leptin level. Results showed that administration of flavonoids from *HS* to lactating dams decreased preweaning and increased postweaning body weights and BMI, increased postweaning food intake of the offspring and decreased leptin concentration in the non-sucrose group whereas it increased leptin concentration in the sucrose group. It is concluded that maternal consumption of flavonoids from *HS* during lactation, with or without sucrose, may predispose the offspring to increased weight gain later in life through increased food intake.

EFFECT OF CONSUMPTION OF AQUEOUS LEAF EXTRACT OF *GONGRONEMA LATIFOLIUM* BY LACTATING WISTAR RATS ON SUCROSE-INDUCED PROGRAMMING OF METABOLIC DYSFUNCTION IN THE OFFSPRING

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Consumption of excess sugar has been implicated in the development of a number of metabolic abnormalities and the potential of herbs such as *Gongronema latifolium (GL)* has been increasingly recognized in prevention and treatment of human diseases. This research investigated the effect of consumption of aqueous leaf extract of *GL* by lactating Wistar rats on sucrose-induced programming of metabolic dysfunction in young adult offspring. 32 adult female rats were used for this study. They were divided into two groups; Non-sucrose treated group and Sucrose treated groups which were further subdivided into eight subgroups of four rats each representing the different concentrations of the extract as follows: Non-sucrose treated subgroup were administered 100mg/kg, 200mg/kg and 400mg/kg while the Sucrose treated groups were administered 30% sucrose with 100mg/kg, 200mg/kg and 400mg/kg of *GL* extract.. The extract was administered orally and daily throughout lactation. At PND 42, blood was withdrawn from both male and female offspring for estimation of serum lipid profile and insulin. Results showed that administration of sucrose with the extracts caused a dose related significant decrease ($P<0.05$) in offspring body weight, BMI, food intake, blood glucose level, decreased the Total cholesterol, triglyceride, LDL-C, VLDL, increased HDL. It was then concluded that consumption of aqueous leaf extract of *GL* during lactation probably has an abating effect on sucrose induced programming of metabolic dysfunction.

Na^+-K^+ PUMP ACTIVITY DIFFERENTIALLY MODULATES REACTIVITY OF ISOLATED RABBIT CAROTID ARTERIES EXPOSED TO ERYTHROCYTE CONSTITUENTS

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The possible involvement of vasospastic mechanisms in vaso-occlusive crisis of sickle cell disease remain unclear. This study aimed to establish if Na^+-K^+ATPase enzyme activity modulates vascular reactivity in response to phenylephrine (PE) in isolated rabbit carotid arteries exposed to erythrocyte constituents. Two (2) mm rabbit carotid arterial ring preparations were placed in 20 ml organ baths containing physiological salt solution (PSS) bubbled with 95% O_2, 5% CO_2, at 37^{o}C and pH 7.4 and isometric contractions measured, under an initial load of 2g. Arterial rings were equilibrated for 60 minutes and exposed to 50 µl of each erythrocyte constituents at an adjusted haematocrit of 0.6. Rings were exposed to K^+-free PSS in the absence (control) or presence of RBC constituents (ghosts, erythrocytes and haemoglobin solution) from Hb SS subjects for 30 minutes and contracted with 10^{-7} M PE. Re-introduction of K^+ (5 mM) to the bath would cause relaxation due to increased Na-K pump activity and hyperpolarization of the membrane. All results are presented as means ± SEM. The percentage

relaxation response to 5 mM K^+ observed in control and in the presence of SS GHOST, SS HBS and SS RBC were 43.28 ± 5.7, 15.46 ± 6.3, 19.77 ± 9.2 and 25.82 ± 8.9 respectively. 5 mM K^+ relaxation was differentially attenuated ($p<0.05$) in the order: SS GHOST > SS HBS > SS RBC. The results suggest a possible impairment of Na^+-K^+ ATPase enzyme activity in SCD, enhanced phenylephrine contractions by exposure to SS GHOST and a possible impairment of endothelial function.

COMPARATIVE EVALUATION OF THE EFFECT TWO ANTI-MALARIA DRUGS (CHLOROQUINE AND ARTEQUIN) ON SOME SERUM BIOCHEMICAL PARAMETERS IN RATS

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Artemisinin combination therapies (ACT) have replaced the old drugs (like chloroquine) used as first line treatment for malaria. This current study aimed to investigate the comparative effects of chloroquine (an old drug) and artequin (an ACT drug) on serum biochemical indices in rats. Thirty-six (36) Wistar rats were randomly assigned into 2 batches. Each batch had 3 groups of 6 rats each. Group 1 was control, groups 2 and 3 respectively received artequin (1.6mg/100g bwt) and chloroquine (0.875mg/100g bwt) orally and once daily. Administration lasted for 3 and 7 days for batches 1 and 2 respectively. The biochemical analysis of the serum was carried out using standard methods. Results obtained on both days 3 and 7 showed that serum total protein and globulin concentrations in the artequin group was significantly lower ($p<0.05$) compared to control. The alkaline phosphatase concentration in the artequin group on day 7 was significantly ($p>0.05$) higher compared to control. In conclusion, administration of artequin and chloroquine at their recommended doses and duration is relatively safe. Prolonged administration of artequin could predispose to low serum proteins and globulin with accompanied elevations in ALP levels while chloroquine could increase AST level signifying hepatocellular damage.

EFFECT OF CIMETIDINE ON HEMATOLOGICAL INDICES OF WISTAR RATS: MODULATORY ROLE OF VITAMIN C

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Cimetidine is a drug used in treatment of dyspepsia which is a clinical condition with widespread distribution. The aim of this study was to evaluate the effect of chronic cimetidine treatment at therapeutic dose on hematological indices and the modulatory role of vitamin C on any change induced by cimetidine treatment. Forty adult male Wistar rats were divided into four groups (n = 10) and treated orally for 60 days with distilled water (control); cimetidine (30 mg kg^{-1}); cimetidine (30 mg kg^{-1}) + vitamin C (25 mg kg^{-1}) and cimetidine (30 mg kg^{-1}) + vitamin C (50 mg kg^{-1}). At the end of the study blood was collected by heart puncture following adequate anesthesia. Total white blood cell (WBC) count (5.99 ± 0.20 x $10^3/mm^3$) and total serum protein (6.44 ± 0.21 g/dl) of the cimetidine-treated group were lower than that of the control (7.95 ± 0.29 x $10^3/mm^3$ and 7.26 ± 0.18 g/dl, respectively), while the value for red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC), color index (C.I) and erythrocyte sedimentation rate (ESR) of the two groups were not significantly different. Treatment with vitamin C prevented the cimetidine-induced decrease in total WBC count and total serum protein. It was concluded that chronic cimetidine administration at therapeutic dose caused a significant decrease in WBC count and serum protein; and no significant effect on RBC count, PCV, Hb, MCV, MCH, MCHC, C.I and ESR; and vitamin C at 25 mg kg^{-1} was more effective than at 50 mg kg^{-1} in reversing the decrease in WBC count and serum protein caused by cimetidine treatment.

EVALUATION OF TOXICITY OF ALUMINIUM-TAINTED WATER IMPACT IN MALE WSTAR RATS: OXIDATIVE STRESS IN HEART AND KIDNEY

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Aluminium is a ubiquitous element; comprising about 8% of the earth's surface; and it has no known beneficial effect in humans, but can enhance adverse health effects. The adverse effect of aluminium on body organs is incompletely understood, necessitating the need to better understand the mechanistic-link in its induced adverse health outcomes.This study evaluated the oxidative stress and established the predictor ratio of the negative impact of aluminium chloride - tainted water ($AlCl_3$) assessed by alterations in pro-oxidant/antioxidant in the heart and kidney of male Wistar rats. Fifty male Wistar rats were randomly assigned to five groups of 10 rats each. Control group was given normal drinking water, while the $AlCl_3$ treated animals were administered 200, 400, 600 and 800mg/kg of $AlCl_3$ orally, once daily for 28 days. Heart and kidney specimens were collected for assessment of oxidative stress markers-malondialdehyde (MDA) and protein carbonyl (PCO) and glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities. The results showed that $AlCl_3$ in a dose-response-organ specificity induced significantly ($P\leq0.05$) higher oxidant/antioxidant enzymes' alterations in rats as reflected by marked increased MDA level with a concomitant decreased GPx, SOD and CAT activity.

Correlation coefficient indicated that all the oxidative stress markers were significantly different upon comparing in both $AlCl_3$ and control groups. In conclusion, our data indicated that dose-response-organ dependent raised oxidative stresses are the possible mechanistic-link in $AlCl_3$ induced male rat cardio-renal toxicity. Additionally, the established predictor ratio of the interplay in the interrelationship of the damaged tissue biomarkers can contribute to evaluation of pathophysiology risk of $AlCl_3$ in heart and kidney.

IN-VITRO NEGATIVE CHRONOTOPIC AND INOTROPIC EFFECTS OF AQUEOUS LEAF EXTRACT OF OCIMUM BASILICUM (SWEET BASIL) ON ISOLATED PERFUSED RABBIT HEART

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Numerous cardiovascular health benefits have been attributed to Ocimum basilicum. The aim of this study is to explore the effect of aqueous leaf extract of Ocimum basilicum on force and rate of contraction of the heart using an isolated perfused heart system. In this study different concentration (10mg/ml, 25mg/ml, 50mg/ml) of the aqueous leaf extract of Ocimum basilicum were used and the experiment was carried out using the isolated perfused rabbit heart model. Standard drugs such as Adrenaline, Propranolol, Acetylcholine and Atropine were also administered and their results were compared with those of the extract. The standard drugs were administered before the extract and at each administration the heart was allowed to recover or reach a basal contraction before the next administration. The extract exerts a negative chronotropic and negative inotropic effect on the isolated heart tissue in a concentration dependent fashion ($p < 0.005$). In conclusion aqueous leave extract of Ocimum basilicum possess negative inotropic and chronotropic properties.

MODULATORY ROLE OF CLOVE SUPPLEMENT ON CHRONIC RESTRAIN STRESS INDUCED ALTERATION IN PAIN PERCEPTION AND INFLAMMATION IN WISTAR RATS.

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The use of herbal medicine is wide spreading and growing, clove is being used for different medicinal purposes such as pain reliever for tooth aches, antiseptic, antispasmodic, antiviral and antibacterial. This study aims to determine the modulatory role of clove supplement on pain perception, inflammation and biomarkers of oxidative stress in Wistar rats exposed to chronic restrain stress. Forty male Wistar rats weighing 100-120g were used for the study. The animals were divided into four groups; positive control group (no stress), negative control group (exposed to restrain stress for four weeks without supplement), third group were given 3% clove supplement with 97% standard feed, fourth group were given 6% clove supplement with 94% standard feed. The third and the fourth groups were both exposed to restrain stress for four weeks (chronic stress). In the tail flick test, clove exhibited more pronounced anti nociceptive effect in the 6% clove supplement group, while a mild reduction in pain response latency at 3% clove supplement group was observed. The result of the anti-inflammatory study revealed that 3% and 6% clove supplement groups possessed anti-inflammatory activity with a maximum inhibitory effect at the end of four hours (4hrs). This study also showed an increase in the serum level of *SOD in the supplement groups, while a decrease in MDA level across the treatment groups was observed. The results obtained from this study confirmed the potentiality of clove as an anti-inflammatory and analgesic agent, as well as a potential anti-oxidant.

ACUTE EFFECTS OF 90-MINUTE FOOTBALL MATCH ON RED BLOOD INDICES IN SEDENTARY YOUNG MALES.

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Previous study, had demonstrated that standard ninety-minute football match did not have adverse effects on blood rheology in regularly trained male footballers. In this study, we aimed to investigate the acute effects of a single 90-minute football match on red blood cell indices in sedentary young males. This quasi- experimental study was carried out on 20 healthy males (20.00±0.48years old). The university institutional review board gave approval for all procedures in accordance with the Declaration of Helsinki. Fasting blood (3ml) was collected from antecubital vein before and after the 90 minutes football match. From the samples, pre- and post-match red blood cell indices were determined using standard methods. Data were analyzed by SPSS version 20 using t student and paired t-tests in order to compare group before and after exercise training. The 90 minutes football match decreased Red Blood Cell count and Packed Cell Volume significantly ($P \leq 0.05$). But it increased the Plasma Volume significantly ($P \leq 0.05$). Results of this study showed that an acute effect of a single 90-minute football match maintains red blood cells and pack cell volume are within physiological range in sedentary young men, thereby optimizing microcirculation to enhanced oxygen delivery to the working muscle.

EFFECTS OF ZINC AND FOLIC ACID ON SPERM MOTILITY AND TUMOUR NECROSIS FACTOR-ALPHA (TNF-A) LEVELS IN TESTICULAR ISCHAEMIC-REPERFUSION INJURY IN WISTAR RATS

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Ischaemic-Reperfusion Injury is a complex phenomenon that induces cell damage through a biphasic process-Ischaemia and reperfusion. The reperfusion phase of this process results in generation of reactive oxygen species and pro-inflammatory cytokines which affect sperm motility. This study attempts to investigate the ameliorative effect of zinc and folic acid on sperm motility and TNF-α levels following IRI. Thirty male Wistar rats were divided into six groups comprising of five animals (n=5). Group 1(control) were given 1 ml/kg normal saline for 21 days. Group 2 (sham) were given normal saline for 21 days followed by sham treatment. Group 3 (torsion/detorsion) were given normal saline for 21 days followed by torsion-detorsion. Rats in Group 4 (folic acid) were treated with (2 mg/kg)folic acid for 21 days followed by torsion-detorsion of the testis. Group 5 (zinc) were treated with (50mg/kg) zinc for 21 days followed by torsion-detorsion .Group 6 (Aspirin) were treated with (200mg/kg) aspirin for 21 days and induced with torsion-detorsion. At the end of 21 days, the epididymises were harvested and sperm motility investigated. Blood samples were collected, the sera harvested and used for TNF-α assay. The number of the non-motile cells decreased significantly ($p<0.05$) in the folic acid and zinc treated groups compared with the control. There was no significant difference in the TNF-α levels in the treated groups but a slight decrease was recorded in the zinc treated group. Results from this study suggest that oral treatment with folic and zinc could significantly reduce the adverse effect of IRI

ALLOXAN-INDUCED DIABETES CAUSES LIVER FUNCTIONS AND LIPID PROFILE CHANGES IN ALBINO WISTAR RATS: ROLE OF ETHNOLIC LEAF EXTRACT OF *GUIERA SENEGALENSIS*

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In most of African countries, a large number of diseases are treated administering plant infusions. Guierasenegalensis (Gs) has been used treats many diseases in Northern Nigeria, but its effects on liver function test, blood glucose level and lipid profile in diabetic rats has not been documented. Thirty five male albino rats with average weight of 200 g-250 g were used for study. They were divided into five groups of seven rats each: Group A (normal control). Group B (diabetic control) Groups C and D and E were induced with diabetes and treated daily with 100mg/kg, 150 mg/kg and 200 mg/Kg body weight of Gs leaf extract respectively for three weeks. At the end of this experimental procedure, rats were anaesthetized with diethyl-ether vapour. Blood samples were collected through cardiac puncture for measurement of serum metabolites. The result demonstrated that administration of ethnolic leaf extract of Gs in the induced diabetes rats has not shown any significant changes in the activities of ALP, AST and ALT when compared with diabetic control. However a significant increase was recorded in the serum total protein (TP) and albumin (ALB) when all the doses of Gs were compared with diabetic control. Administration of ethnolic leaf extract of Gs to alloxanized diabetic rats at the doses considered possesses hypoglycemic activities.

EFFECTS OF CALCITRIOL SUPPLEMENTATION ON RENAL, LIVER AND LIPID PEROXIDATION BIOMAKERS, IN FRUCTOSE-DRINKING ALBINO WISTAR RATS

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Type 2 diabetes mellitus is an important public health problem. The study was designed to examine the effects of oral calcitriol treatment on indices of renal and liver failure, antioxidant status and lipid peroxidation in fructose-drinking rats. Animals (130- 200 Kg) were randomised into four groups of five rats each and subjected to 125 µg/Kg body weight calcitriol treatment for three weeks after five weeks of fructose drinking. *Group I:* Control; normal rat feed + distilled water, *Group II:* normal rat feed + 125 µg/Kg body weight of Calcitriol, *Group III:* normal rat feed + 10% fructose solution, *Group IV:* normal rat feed + 10% fructose solution + 125 µg/Kg body weight of Calcitriol. All the parameters were determined using available commercial kits. Results showed that fructose-drinking rats exhibited significant increased in urea, creatinine, liver enzymes activities and lipid peroxidation index while the activities of Superoxide dismutase (SOD) and Catalase (CAT) were significantly reduced compared with the control. Calcitriol treatment significantly reduced renal failure indicators and activities of liver enzymes. However the activities of antioxidant enzymes were significantly increased. it was contrived that calcitriol treatment exhibited a reno-hepatic protection, enhanced the activities of the antioxidant enzymes and prevented lipid peroxidation in fructose-drinking rats.

THE EFFECT OF ADMINISTRATION OF CURCUMIN DURING SUCKLING ON THE DEVELOPMENTAL PROGRAMMING OF METABOLIC FUNCTION IN MALE AND FEMALE ADOLESCENT SPRAGUE DAWLEY RATS

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The current global epidemic of metabolic syndrome is linked to the increased consumption of fructose rich diets. Nutritional perturbations with natural polyphenols during the suckling period have demonstrated beneficial metabolic programming effects. Curcumin is a dietary spice that has anti-diabetic, antioxidant and anti-inflammatory properties. We aimed to determine whether administration of curcumin to rats during suckling would protect or predispose them to the adverse effects of a post weaning high fructose diet. Male and female Sprague Dawley pups (n=128) were allocated to four treatment groups and administered either a 0.5% dimethyl sulfoxide solution, curcumin (500mg.kg^{-1}), fructose (20%, w/v) or a combination of curcumin and fructose daily via oral gavage from postnatal days 6 to 21. All the rats were weaned unto normal rat chow and each of the initial groups was further subdivided into two subgroups; one group had plain tap water while the other had fructose (20%, w/v) as their drinking solution for six weeks. The rats were then fasted overnight and euthanised on postnatal day 63. Blood was collected via cardiac puncture and used for metabolic substrates and hormonal assays. There were no differences ($p>0.05$, ANOVA) in the fasting blood glucose, triglycerides, cholesterol, plasma concentrations of insulin, adiponectin and the homeostatic model of insulin resistance across the treatment groups. Administration of curcumin during suckling, a critical window of developmental plasticity in the rat, did not affect the metabolic response of adolescent rats to a post weaning high fructose diet.

EVALUATION OF THE ROLE OF ELLAGIC ACID ON MOTOR AND OXIDATIVE RESPONSES IN PENTYLENETETRAZOLE-KINDLED RATS

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Epilepsy is a neurologic disorder affecting more than 50 million people worldwide. Motor function impairment is among the disorders caused by epilepsy. Oxidative stress plays part in epileptic kindling. This study assessed the motor strength role of ellagic acid in pentyleneterazole (PTZ)-kindled rats. Thirty male Wistar rats (200 – 300g) with 6 groups of 5 rats each were used. Groups 1 – 5 received 35mg/kg PTZ, while group 6 received distilled water (*s.c*) on alternate days. One hour before PTZ administration, group 2, 3 and 4 received 15, 30 and 60 mg/kg (*p.o*) ellagic acid dissolved in 10% Dimethylsulphoxide (DMSO) respectively while group 5 received 30mg/kg phenobarbital (*i.p*) and were observed for seizure activity after PTZ injections. When kindling was achieved, motor strength test was conducted after which the rats were anaesthetized and their brain tissues used for assaying malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Results showed no significant ($P < 0.05$) improvement in motor strength in all the treated groups. A significant decrease ($P < 0.05$) was observed in MDA concentration in the treated groups when compared to control group 1. Also SOD increased significantly ($P < 0.05$) in all the groups when compared to control group 1. GPx was significantly increased ($P < 0.05$) in group 4 when compared to group 1 control. Findings from the study showed that epileptic seizure did not affect motor strength while oxidative stress was involved in kindling. Ellagic acid may be a potent antiepileptic drug with no associated side effects.

EVALUATION OF THE EFFECTS OF *CANNABIS SATIVA* L. EXTRACT ON MOTOR FUNCTION OF MALE WISTAR RATS

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The present study was aimed at investigating the effect of *Cannabis sativa* L. administration on motor coordination activity of adults Wistar rats. The work was limited to motor activity test of the cerebral cortex of adult male Wistar rats. Fifteen male adults Wistar rats of average weight 220g were divided into 3 groups with 5 animals per group. Animals in group 1(control) were given distilled water, while group 2 and 3 were administered with 250mg/kg and 500mg/kg b.wt respectively via oral route, daily for 21 days. Motor exploratory activity was assessed using Ladder rung walking test method. Motor assessment showed a significant increase ($P<0.05$) in the meantime taken to reach the home cage in groups 2, and 3 when compared to the control. It can therefore be concluded that *Cannabis sativa L.* administration result in motor exploratory impairment which could be due to degenerative changes in the cerebral cortex of adults Wistar rats.

THE HYPOTENSIVE EFFECT OF *HIBISCUS SABDARIFFA* TEA MAY OCCUR THROUGH THE INHIBITION OF THE DISCHARGE OF THE SYMPATHETIC NERVOUS SYSTEM

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This study tested the hypothesis that the hypotensive effect of *Hibiscus sabdariffa* tea (HST) may be due to its

attenuation of discharge of the sympathetic nervous system (SNS). Using a double-blinded placebo-controlled design, the hand grip exercise (HGE) was used to activate the SNS.[1] Following ethical approval, HGE was performed in healthy human subjects before and after the oral administration of 200mg/kg HST (n=20) or food colourant (n=20) which served as placebo. The basal blood pressure (BP) and pulse rate (PR) were obtained, then each subject held the hand-grip dynamometer forcefully at 30% maximal voluntary contraction (MVC) for 1-2 minutes until the onset of fatigue, and the BP and PR responses were measured. The mean arterial pressure (MAP) was taken as representative BP. Results were expressed as Mean±SEM and $P<0.05$ was considered significant. In the presence of HST, the HGE-induced changes (ΔMAP=8.7±1.3mmHg; ΔPR=8.4±1.0 beats/min) were significantly reduced compared to its absence (ΔMAP=15.0±1.8mmHg, ΔPR=14.5±1.5 beats/min; $P<0.0001$ respectively). However, in the presence of the food colourant, these changes (ΔMAP=11.2±0.6mmHg, ΔPR=11.9±0.8 beats/min) were significantly higher compared to its absence (ΔMAP=8.7±0.7mmHg, ΔPR= 9.7±0.7 beats/min; $P<0.0001$ respectively). These results suggest that HGE-induced activation of the SNS was attenuated by HST but aggravated by the food colourant. It is concluded that the hypotensive effect of HST may occur through the inhibition of SNS activation while the food colourant may further activate it.

EFFECT OF CIGARETTE SMOKING ON COGNITIVE FUNCTION AMONG YOUNG MALE UNDERGRADUATES IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

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About 2 billion people worldwide smoke tobacco product mostly in form of cigarette. Evidences on the effects of cigarette smoking on cognition have been very conflicting. While some studies have shown cigarette smoking to be protective against cognitive impairments other studies have shown otherwise. The aim of this study is to assess the relationship between cigarette smoking and cognitive impairment in young male undergraduate students of Ahmadu Bello University, Zaria, Nigeria. This is a cross-sectional study, comprising 62 smokers and 41 never-smokers as volunteers. Each volunteer was randomly selected and subjected to four cognitive battery tests which include the mini-mental state examination (MMSE), clock-drawing test (CDT), trail-making test (TMT) and verbal fluency test (VFT), with an inter-test period of about 15 minutes. Analysis was done using the SSPS v17. Most of the volunteers were within 18-27 years of age (50 [80.6%] of smokers and 35 [85.4%] of never-smokers). On individual cognitive battery test, varying degrees of cognitive impairments were found among both groups: MMSE (3.2% of smokers, none of never-smokers); VFT 1 (9.7% of smokers, 9.8% of never-smokers); VFT 2 (32.3% of smokers, 17.1% of never-smokers); CDT (38.7% of smokers, 14.6% of never-smokers); TMT A (71.0% of smokers, 75.6% of never-smokers) and TMT B (46.8% of smokers, 51.2% of never-smokers). Overall, the never-smokers appear to performed better than the smokers except in the cognitively-tasking TMT but only 16.1% of the smokers had cognitive impairment, as against 26.8% (11) of the never-smokers. Chi-square analysis revealed no association between smoking and cognitive impairment. Pearson's correlation also revealed weak correlations between smoking and cognitive impairment in all the tests. Therefore, light to moderate cigarette smoking confers some protective effect against cognitive impairment probably due to the cognitive-enhancing effect of nicotine contained in the cigarette smoke.

THE AWARENESS OF PHYSIOLOGY AS A SCIENTIFIC DISCIPLINE AND A PROFESSION AMONG SENIOR SECONDARY SCHOOL STUDENTS IN ABUJA, NIGERIA

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The recognition of Physiology as a profession and major discipline in medicine and scientific research in Nigeria is gaining momentum. This attention is however low especially among prospective university applicants. This study was conducted to determine the level of awareness of Physiology among secondary school students in the six local government areas of Abuja. The research was carried out in twelve randomly chosen secondary schools (private and public) in Abuja, Nigeria. One hundred and twenty (n=120) senior secondary school students (SSI-SSIII) were asked to fill in questionnaire, of which ninety-eight (98) responded. The first part of the survey included personal data and brief history. The second part contained 9 close-ended questions assessing students' knowledge about physiology as a discipline. The results were treated statistically using student's t-test. Apparently, data from the respondents shows that private and public schools are not familiar with the varied facets of Physiology as a discipline. Among the students in the private school, a mean of 2.18 for male and 2.29 female were obtained; while in public schools, a mean of 2.43 for male and 2.52 for female were obtained respectively. However, all the respondents were aware that there are professionals called Physiologist (private school: Mean $_{male}$ = 3.5; Mean $_{female}$ = 4.31 and public schools: Mean $_{male}$ = 3.0; Mean $_{female}$ = 4.31).The results of this study shows that most senior secondary school students are not very familiar with Physiology as a scientific discipline. However, their knowledge of Physiology as a profession was remarkable.

MODULATORY EFFECTS OF ACETONE EXTRACT OF Combretum micranthum BARK ON CYCLOOXYGENASE ENZYME

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Inflammation is an essential response provided by the immune system that ensures the survival during infection and tissue injury. Inflammatory responses are essential for the maintenance of normal tissue homeostasis. Combretum micrathum is used traditionally in Senegal and Mali for fatigue, liver ailments, headache, convalescence, diabetes, blood disease, weight loss, cancer, sleep disorders, most of this diseases are associated with inflammation. The aim of this study was to validate the numerous uses of Combretum micranthum plant. The acetone extract of Combretum micranthumwas screened for cyclooxygenase (COX) activity in a 96 well microtitre plate using COX screening assay kit. The result obtained demonstrated a weak inhibitory activity by the extract on both COX-1 and COX-2 with median inhibitory concentration values of 52.45±3.4µg/ml and 66.01±4.7µg/ml respectively. This shows that the extract had a weak anti-inflammatory property which may be attributed to the presence of some phytochemicals such as resins and glycosides in the extract. The median lethal concentration (LC50) was 41.32±0.92µg/ml which signifies low toxicity compared to the control drug doxorubicin with an LC50 value of 3.8±0.08µg/ml. In conclusion, the acetone extract of cambretum micranthum bark was found possess weak anti-inflammatory activity and low toxicity to vero cells.

LIVER ENZYMES AND SERUM ELECTROLYTES EVALUATION IN FEMALE LACTATING WISTAR RATS FOLLOWING FERMENTED SOYA BEAN AND ASCORBIC ACID SUPPLEMENTATION

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In mammals, lactation is the most energetically demanding period of a female's reproductive life. However, some of the substances used to enhance lactation have side effects. This study was designed to investigate the effect of fermented Soya bean and ascorbic acid supplements on some liver enzymes and histology. At parturition, the animals were randomly divided into seven (7) groups of five (5) rats each (n=5) and treated as follows: Group I: (Normal control) was given normal feed and distilled water, orally (1 ml/kg bw), Group II: metoclopramide (5 mg/kg bw), Group III: 100 mg/kg bw of Vitamin C, Groups IV, V and VI were given soya bean supplement thus; 10%, 20% and 40%, respectively. Group VII was co-administered with 20% soya bean supplement and Vitamin C (100 mg/kg bw). Treatment was done for the period of ten (10) days at 06:00 hours daily. The result on serum ALP level showed a significant increase in all the supplements treated groups; SB 10% (110.80 ± 1.63), SB 20% (127.60 ± 9.60), SB 40% (122.80 ± 2.60) and SB 20% + VIT C (129.40 ± 4.90) compared to the controls (86.60 ± 3.73) ($P< 0.05$). Although there was increase in the level of serum calcium, sodium, magnesium, potassium, chloride and bicarbonate in all the treated groups, it was however not significant compared to the control and metoclopramide treated group. The co-administration of the soya bean supplement and Vitamin C showed a significant increase in serum ALP, ALT and AST levels which could infer a detrimental potential of such combination to the hepatocytes.

EFFECTS OF HYDROMETHANOL EXTRACTS OF *Garcinia kola* ON SOME BIOCHEMICAL PARAMETERS OF MALE WISTAR RATS.

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Garcinia Kola is commonly used in traditional medicine for the treatment of diverse ailments including coronary artery diseases. Thus, this study aims to determine the effect of hydromethanol (1:4) extracts of the pulp and seed coat of *Garcinia kola* on serum lipid profile and its antioxidant properties. The two forms were separately dried and blended to powder. Forty male wistar rats (8 per group) were assigned into Five (5) groups. Groups were treated thus: Group one; control. Group two; 100mg/kg pulp extract. Group three; 200mg/kg pulp extract. Group four; 100mg/kg seed coat extract. Group five; 200mg/kg seed coat extract; for 30 and 60 days duration. On treatment conclusion, blood was collected for the determination of lipid profile and antioxidant properties. The higher dose of the pulp and seed coat extracts significantly ($P<0.05$) increased the catalase level and superoxide dismutase enzyme activity, whereas, both the higher and lower doses of the seed coat extract caused a reduction in malondialdehyde level. The serum total cholesterol was significantly elevated by the higher dose of the pulp extract while the seed coat extract caused significantly increased high density lipoprotein cholesterol level and a reduction in the low density lipoprotein level. The two extracts demonstrated marked antioxidant effects. The seed coat of *Garcinia kola* may possess the potential to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The seed coat extract may also be useful in preventing coronary artery disease and other atherosclerotic problems.

BLOOD GLUCOSE AND OXIDATIVE STRESS BIOMARKERS ASSESSMENT IN ALLOXAN INDUCED DIABETIC WISTAR RATS TREATED WITH QUERCETIN

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The study was designed to evaluate the effect of Quercetin on blood glucose level and oxidative stress biomarkers in Alloxan-induced diabetic wistar rats. The animals were divided into six groups of five each (n=5). Group I served as control and received distilled water, group II received dimethylsulphoxide 1% (DMSO), group III and IV received Quercetin at 50mg/kg and 100mg/kg respectively. Group V received vitamin C (100mg/kg) while group VI was given glibenclamide (1 mg/kg). Diabetes was induced by injection of alloxan 150 mg/kg intraperitoneally. All administrations were done via oral gavage for 21 days. Quercetin at a dose of 50mg/kg and 100mg/kg significantly (P<0.05) reduced fasting blood glucose level when compared to control (101.00 ± 7.27 vs 122.56 ± 8.02 mg/dL) vs(332.80mg/dL ± 36.53mg/dL). Serum catalase was significantly higher in the quercetin treated groups compared to the diabetic control. Serum level of SOD was significantly higher in the 100 mg/kg quercetin treated group relative to the diabetic control 2.44 ± 0.07 and 2.10 ± 0.05 vs 0.92 ±0.05. Serum malondialdehyde concentration was significantly lower (P<0.05) in the groups treated with qurcetin compared to the diabetic control 0.98 ± 0.01 vs 1.32 ± 0.06,. In conclusion oral administration of quercetin has been found to reduce blood glucose level, increase serum antioxidant level and significantly decrease serum malondialdehyde level in Alloxan-induced diabetic wistar rats.

THE EFFECT OF AQUEOUS AND N-HEXANE EXTRACTS OF *NIGELLA SATIVA* IN ALUMINIUM CHLORIDE –INDUCED HEMATOLOGICAL ALTERATION AND OXIDATIVE STRESS.

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Aluminium Chloride ($AlCl_3$) is a neurotoxic substance that has been known to induce hematological alteration and oxidative stress. The present study was designed to investigate the protective effect of *Nigella sativa* against $AlCl_3$ induce hematological alterations and oxidative stress in adult Wistar rats. Thirty adult Wistar rats divided into six groups of five animals each. Group 1 (control group) was given Tween 80%; Group 2 was given Aqueous *Nigella sativa* (ANS) extract at dose of 200mg/kg; Group 3 was given Hexane *Nigella sativa* (HNS) extract at dose of 50mg/kg ; Group 4 was given 900mg/kg of $AlCl_3$; Group 5 was given 200mg/kg ANS + 900mg/kg $AlCl_3$ and Group 6 was given 50mg/kg HNS extract + 900mg/kg $AlCl_3$. $AlCl_3$ significantly (p<0.05) decreased RBC count but showed a significant (p<0.05) increased in PCV, HB, MCH, and MCHC. Treatment with HNS and ANS reversed the effects of $AlCl_3$. But, group treated with HNS alone significantly (p<0.05) increased RBC count. Lymphocytes and WBC counts were significantly (p<0.05) decreased by $AlCl_3$, which was reversed with HNS. Treatment with either of the extract alone significantly (p<0.05) increased lymphocytes count. $AlCl_3$ significantly (p<0.05) decreased the level of MDA, and GSH as well as SOD and CAT activities. Treatment with ANS showed no significant (p<0.05) effect on MDA and GSH but significantly increased (p<0.05) catalase activity, whereas treatment with HNS significantly (p<0.05) decreased MDA level only. It can be concluded that extracts of *Nigella sativa* increased hematological parameters and alleviate oxidative stress in Wistar rats treated with $AlCl_3$

EFFECTS OF HYDROMETHANOL EXTRACTS OF *Garcinia kola* ON SOME BIOCHEMICAL PARAMETERS OF MALE WISTAR RATS.

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G*arcinia Kola* is commonly used in traditional medicine for the treatment of diverse ailments including coronary artery diseases. Thus, this study aims to determine the effect of hydromethanol (1:4) extracts of the pulp and seed coat of *Garcinia kola* on serum lipid profile and its antioxidant properties. The two forms were separately dried and blended to powder. Forty male wistar rats (8 per group) were assigned into Five (5) groups. Groups were treated thus: Group one; control. Group two; 100mg/kg pulp extract. Group three; 200mg/kg pulp extract. Group four; 100mg/kg seed coat extract. Group five; 200mg/kg seed coat extract; for 30 and 60 days duration. On treatment conclusion, blood was collected for the determination of lipid profile and antioxidant properties. The higher dose of the pulp and seed coat extracts significantly (P<0.05) increased the catalase level and superoxide dismutase enzyme activity, whereas, both the higher and lower doses of the seed coat extract caused a reduction in malondialdehyde level. The serum total cholesterol was significantly elevated by the higher dose of the pulp extract while the seed coat extract caused significantly increased high density lipoprotein cholesterol level and a reduction in the low density lipoprotein level. The two extracts demonstrated marked antioxidant effects. The seed coat of *Garcinia kola* may possess the potential to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The seed coat extract may also be useful in preventing coronary artery disease and other atherosclerotic problems.

SERUM LACTOGENIC HORMONES AND TOTAL MILK YIELD IN FEMALE LACTATING WISTAR RATS TREATED WITH EXTRACT OF *CITRULLUS LANATUS SEEDS*

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Around the world few herbs used for lactation purposes have not been scientifically evaluated but their traditional use, suggests and some beneficial effects. This study was designed to evaluate the lactogenic effect of *Citrullus lanatus* seeds (extract) in female lactating Wistar rats. Twenty apparently healthy female Wistar rats weighing between 160-200 g were randomly grouped into four (4) of five animals each (n=5) and treated orally for a period of ten (10) days, starting from day 3 after parturition. Group 1: Control (2 ml/kg) of distilled water; Group 2: Metoclopramide (5 mg/kg); Group 3: Extract (200 mg/kg); Group 4: Extract (400 mg/kg). Total milk yield was obtained from the difference between pre and post suckling pups weight. At the end of the treatment, rats (dams) were anaesthetized using diazepam and ketamine injection (75 and 25 mg/kg) respectively, and the blood samples obtained via cardiac puncture for biochemical analysis. There was a non-significant increase in serum prolactin level in all the treated groups when compared to control. Serum oxytocin level increased significant ($P< 0.05$) in groups 2, 3 and 4 compared to control. There was also a significant increase ($P< 0.05$) in serum oxytocin level in group 3 compared to group 2. Total milk yield increased significantly ($P< 0.05$) in the group 2 and 3 compared to control. In conclusion, *Citrullus lanatus* was found to increase oxytocin and milk yield in lactating Wistar rats.

ASSESSMENT OF LIPID PEROXIDATION AND SOME ANTIOXIDANT ENZYMES IN FEMALE LACTATING WISTAR RATS FOLLOWING ASCORBIC ACID AND α-TOCOPHEROL SUPPLEMENTATION

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Oxidative stress, an imbalance between the generation of reactive oxygen species/ reactive nitrogen species and antioxidant defence capacity of the body, is actively involved in the pathogenesis of diabetes and its complications. This study was designed to evaluate lipid peroxidation and antioxidant enzymes in female lactating rats. At parturition, the animals were randomly divided into five groups thus; Group I: (Normal control) was given normal animal feed and distilled water, (1 ml/kg), Group II: metoclopramide (5 mg/kg bw), Group III: 100 mg/kg bw of Vitamin E. Group IV: 100 mg/kg of Vitamin C, whereas Group V was treated with the co-administration of vitamin E and C. Administration was carried out orally for a period of ten (10) days at 06:00 hours daily and the animals were euthanized at the end of the experiment. Serum levels of MDA in group treated with ascorbic acid was significantly decreased ($P< 0.05$) compared to control, metoclopramide treated and α-Tocopherol treated groups. There was also a significant decrease in the grroups treated singly with vitamin C and α-Tocopherol when compared to the group co-administered with ascorbic acid and α-Tocopherol. However there was no statistically significant difference observed in the serum level of Superoxide dismutase (SOD) and Catalase (CAT). Serum level of Glutathione peroxidase (GPx) was statistically significant ($P< 0.05$) in the group administered α-Tocopherol compared to the control. In conclusion, ascorbic acid and α-Tocopherol administered singly was more efficient in alleviating lipid peroxidation than their co-administration.

DETERMINATION OF CORRECTED QT INTERVAL (QTC) AMONG PSYCHIATRIC PATIENTS AT DAWANAU PSYCHIATRIC HOSPITAL, KANO

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Psychiatric patients are often associated with electrocardiographic (ECG) abnormalities notable among which is prolonged QTc which in some cases are life threatening due to the effect of anti psychotic drugs they are placed on. This study aims to determine the QTc of patients on anti psychotic drugs and find out if there are abnormalities in the ECG tracings as documented in the literature. Anthropometric and clinical data were obtained from 323 participants attending Dawanau Psychiatric hospital between April to May 2017. Apparently normal individuals who were not taking anti psychotic drugs were used as controls. ECG was recorded using DEC G-03A 12 Lead ECG machine on all the patients lying supine on a couch. The mean age of the participants was 34.5±9.57 years with 182 (56%) being males and 141 (44%) females. Independent sample t-test among the subjects and controls for BMI, SBP and Heart rate were 21.4±4.4kg/m_2 and 21.57±3.57kg/m_2 (p=0.89), 111.42±16.67mmHg and 124.5±12.47mmHg (p=0.001), and 81.53±17.95 b/min and 73.37±12.03b/min(p=0.015) respectively. The mean corrected QT interval (QTc) was 413.51±30.09ms for the subjects and 391.47±18.38ms for the controls which is statistical significant (p=0.001). Nineteen (6%) of the subjects have prolonged QTc as against none of the controls, Correlation analysis within the subjects indicated no significant association between QTc and duration of anti-psychotic drugs treatment(r=1). It can be concluded that antipsychotic drugs do not affect QTc duration. It is therefore recommended that baseline ECG screening be carried out on all patients on anti-psychotic drugs to help identify those with salient features of myocardial diseases so as to institute concurrent treatment in order to minimize the risk of fatal complications.

LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES ASSESSMENT IN ALLOXAN MONOHYDRATE INDUCED HYPERGLYCAEMIC MALE WISTAR RATS FOLLOWING ORAL ADMINISTRATION OF THEOPHYLLINE AND GLIBENCLAMIDE

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The currently available anti-diabetic drugs are far from being satisfactory. Hence it is therefore imperative that the effects of other drugs like theophylline on blood glucose be studied.This study was designed to evaluate the effect of theophylline and glibenclamide treatment on lipid peroxidation (serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase, glutathione peroxidase and Catalase) in alloxan induced hyperglycaemic male Wistar rats. Thirty healthy male wistar rats weighing between 160-180g were grouped into five of six animals each (n=6) and treated for a period of fourteen days (14) after induction of hyperglycaemia using alloxan monohydrate. Group 1: (Normoglycaemic) Group 2: Diabetic control (DC), Group 3: Glibenclamide, 5mg/kg, Groups 4 and 5; theophylline 5mg/kg and 10mg/kg respectively. At the end of the fourteen (14) days, rats were anesthetized using ketamine and diazepam at 75 and 25 (mg/kg) respectively. Blood samples were taken from all treated groups for evaluation of serum MDA, SOD, GPx and CAT level. The result on serum MDA concentration was significantly decreased ($P< 0.05$) in glibenclamide treated group compared to diabetic control; 1.14±0.03 vs 1.32±0.06. Although a decrease was observed in the theophylline treated groups, the difference was however not statistically significant compared to diabetic control. There was also significant increase ($P< 0.05$) in serum SOD and CAT level in the glibenclamide and theophylline treated group (5 mg/kg) compared to DC; 2.02±0.04 and 1.92±0.24 vs 0.92±0.05 respectively for serum SOD and 53.20±0.58 and 52.80±1.07 vs 46.00±0.84 respectively for CAT. However, serum GPx increased significantly ($P< 0.05$) only in the theophylline treated groups compared to DC. In conclusion, Theophylline and Glibenclamide decreases lipid peroxidation while increasing serum antioxidant levels in alloxan induced hyperglycaemic male Wistar rats after 14 days oral administration

PREVALENCE OF MALARIA PARASITE (PLASMODIUM PARASITE) IN PATIENTS WITH SICKLE CELL ANAEMIA IN CRISIS STATE ATTENDING HAEMATOLOGY CLINICAL OF AHMADU BELLO UNIVERSITY TAECHING HOSPITAL ZARIA, NIGERIA.

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The sickle cell gene has a geographical distribution that is identical to plasmodium species hence its prevalence in the sub-Saharan Africa including Nigeria where malaria is endemic (Edington and Laing, 1957). The high incidence of the sickle cell gene in malaria holoendemic areais attributable to the protection it affords the bearer as explained by the concept of balance polymorphism (Serjeant andSerjeant,2001). Approximately, 300 million people world-wide are affected by malaria. It remains the major course of premature death, Abortion and still birth in the tropic and sub tropic, average of 1 – 1.5 million people die from it every year (WHO, 2003). Malaria is the most common cause of outpatient hospital visit in Nigeria and it consistently ranked among five most common cause of death for all ages (FMOH). This is a cross sectional study involving 30 consenting patients with SCA in crisis which was conducted in the haematology clinic of Ahmadu Bello University Teaching Hospital Zaria. All participants were interviewed using a structured questionnaire and examined clinically, consisting of 17 females (56.7%) and 13 males (43.3%). The ages ranges from 18 to 35. Malaria parasites were found in four (13.7%) patients with SCA in crisis. Females are less protected from malaria than males with incidence of 3 (13.7%) and 1 (7.7%) respectively. Males had more frequency of crisis while female had lower haematocrit level meaning that anaemia is worse in females. Blood transfusion and malaria are positively related. The prevalence of malaria in crisis is 13.7&% females are less protected from malaria than males. The have frequent crisis while the female have lower haematocrit level meaning that anaemia is worse in female. Blood transfusion and malaria are positively related.

COMPARATIVE STUDY OF OSMOTIC FRAGILITY TEST AND MALONDIALDEHYDE CONCENTRATION IN DEXAMETHASONE AND STREPTOZOTOCIN AS A MODEL OF TYPE 2 DIABETES IN HIGH FAT DIET FED RAT

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Different models have been experimented for easy generation of type 2 diabetes interval time. In order to further clarify the pathophysiology of this metabolic disease, this research was done to compare the effect of erythrocyte osmotic fragility (OFT) and malondialdehyde (MDA) concentrations in streptozotocin with high fat diet (STZ/HFD) and dexamethasone with high fat diet (DEXA/HFD) in Wister rats. For the study, 25 male rats of uniform weight and age were selected and divided into five groups, viz, Control group, dexamethazone (DEXA) 2ml/kg, high fat fed rats (HFD), (STZ 30mg/kg/HFD), and (DEXA 0.5ml/kg/HFD). At the end of the study, 5ml of blood of each animal was collected through cardiac puncture; 3ml was put in EDTA bottle and the remaining 2ml in plain tube for serum extraction which were subjected to OFT and MDA concentration analysis. Data obtained were analysed using ANOVA followed by Tukey's post-hoc test for descriptive analysis. The results showed significant increase in OFT for DEXA/HFD (38.20 ±0.86, 49.40 ±0.51, 68.60 ±2.71, 87.20 ±2.44 and 98.00 ±2.00 at 0.7%, 0.6%, 0.5%, 0.4% and 0.3% concentration of NaCl respectively) and STZ/HFD (37.40 ± 2.38, 57.00 ± 5.21, 73.20 ± 5.28, 82.40 ± 2.98, 91.40 ± 2.73 at 0.7%, 0.6%, 0.5%, 0.4% and 0.3% concentration of NaCl respectively) when compared with control group. Also for MDA serum concentration, both STZ (30.14 ± 1.63* nmol/ml) and DEXA (29.56 ± 0.30* nmol/ml)

showed statistical significant increase compared to control group. Conclusively, our findings indicate that rats treated with streptozotocin in combination with high fat diet and dexamethasone in combination with high fat diet are effective way to induce diabetes in Wister rat

PROTECTIVE EFFECT OF CAMEL MILK ON LIVER ENZYMES OF HIGH FAT DIET FED WISTAR RATS

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Camel milk exhibits antioxidant and insulin-like activities beneficial in diabetic mellitus. The study was aimed at investigating the protective effect of camel milk on activities of liver enzymes in diabetic Wistar rats. A total of 20 Wistar rats of both sexes used in the study were divided into four groups: Group I served as control; Group II, positive control; and Groups III and IV administered with camel milk orally at the dose rate of 2 and 4 ml/kg respectively for six weeks. Diabetes mellitus was induced in the rats by high fat diet, containing 10% of oil, 20% mill and 1% cholesterol. Aspartate aminotransferase activity was lower in diabetic rats administrated with camel milk at both 2 ml/kg (9.67 ± 1.93 IU) and at 4 ml/kg (7.97 ± 1.83 IU) than in diabetic group (90.2 ± 2.90 IU). Alanine aminotransferase activity was lower ($p<0.05$) in diabetic rats, administrated with camel milk at 4 ml/kg (6.48 ± 0.42 IU), compared to that of diabetic group (7.73 ± 0.98 IU). The concentration of Alkaline phosphate was lower ($p<0.05$) in diabetic rats, administrated with camel milk at the dose rate of 2 ml/kg (33.90 ± 3.00 IU) and 4 ml/kg (39.30 ± 8.00 IU) than that of the diabetic control (117.83 ± 15.70). Concentrations of total protein and albumin did not differ among the groups treated with camel milk. The result showed that camel milk reduced activities of the liver enzymes and may be beneficial in offering protection against diabetes mellitus. It was concluded that camel milk exerted protective effect against type 2 diabetes mellitus, and may be beneficial in its management.

CALABASH CHALK CHRONIC DIET CONSUMPTION ELEVATES ANXIETY AND PAIN PERCEPTION IN MICE

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Consumption of calabash chalk is a common practice in Nigeria as well as other parts of Africa, especially among pregnant women. Nevertheless, calabash chalk contains lead (Pb) and arsenic which are thought to be harmful to the brain and responsible for cognitive dysfunction. It is therefore conceivable that calabash chalk consumption may affect other neuronal activities in the body such as anxiety and pain. Therefore, this present research study investigated the effects of consumption of this form of pica on anxiety and pain perception in mice. Forty-five (45) Swiss white mice of mixed sex were randomly assigned into 3 groups of 15 mice each. Group 1 served as control, while groups 2 and 3 received low and high doses of calabash calk diets respectively. Feeding lasted for 30 days. Anxiety levels of the mice were assessed with the aid of elevated plus maze and light-dark transition box as well as elevated plus maze, while response to pain stimuli were studied using hot plate and formalin tests. The results showed that the calabash chalk diet-fed mice had significantly increased ($p< 0.05$) close arm duration and stretch attend posture compared to control. Pain perception was significantly increased in the calabash chalk diet-fed mice compared to control. Consumption of calabash chalk elevates anxiety and pain perception in mice. These actions may be as a result of its lead and arsenic content.

ASSESSING THE IMPACT OF RESTRAINT STRESS DURATION ON SOME SPERM INDICES AND OXIDATIVE STRESS BIOMARKERS OF MALE WISTAR RATS

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This experiment was aimed at determining the possible timing, which restraint stress induces oxidative damage and alters some sperm indices among Wistar rats. Animals were randomly divided into three groups of three animals each (n=3).Group I -normal control (undisturbed), Group II-(3 h stress) group, Group III- (6 h stress group). Restraint stress was induced by placing rats in specially constructed restraint meshes for both 3 and 6 hours (between 9.00-15.00 h) for 21 days. Testes homogenate were evaluated for Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH). 3 h stress group showed statistical ($P < 0.05$) decrease in sperm concentration and motility when compared to normal control group and 6 h stress group. However there was statistical ($P < 0.05$) increase in SOD and CAT in normal control groups when compared to stress groups. Restraint stress for both 3 and 6 h induced oxidative stress which might have led to decrease in sperm motility and concentration; however 3 h of stress induced more oxidative damage among male Wistar rats.

ABORTIFACIENT EFFECT OF AQUEOUS LEAF EXTRACT OF *AZADIRACHTA INDICA* (MELLACEAE) IN PREGNANT FEMALE RATS

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Rapid rise in population has caused serious problems in the economic and social growth of human development in countries like Nigeria, leads to poverty. About 80% of the world's population rely on herbal medicinal products as a primary source of medicine. The Abortifacient effect of *Azadirachta indica* was investigated by evaluating the serum estrogen and progesterone level in female pregnant Wistar rats. Female Wistar rats in their pro-estrous phase were cage with male in ratio 2:1. Rat exhibiting thick clump of spermatozoa in their vaginal smear were separated and that is day one of pregnancy.Total of twenty (20) female pregnant Wistar rats were randomly divided into four group of five rats each (n=5). Group I received (control) 1 ml/kg normal saline, Group II received 200 mg/kg Misoprostol, Groups III and IV received 250 mg/kg and 500 mg/kg of the aqueous extract of *Azadirachta indica* respectively. Misoprostol and aqueous extract of *Azadirachta indica* were administered orally on day 4th and day 5th of conception. The results showed that all rats in group I carry their pregnancy to term (delivered). The rats in groups II, III, and IV had their pregnancy aborted. Serum estrogen and progesterone level in aqueous extract treated groups when compared with control group showed a significant decrease. Also, the results for serum estrogen and progesterone in the aqueous extract treated groups when compared with Misoprostol group also showed no significant decrease. *Azadirachta indica* exhibit abortifacient effect as it caused abortion and decreases progesterone level which is the hormone that maintain pregnancy.

POSSIBLE ANTIOXIDANT EFFECTS OF *THEOBROMA CACAO L.* STEM BARK ON PHENYLHYDRAZINE-INDUCED ANEMIA IN WISTAR RATS

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Cocoa (*Theobroma cacao* L.) is a rich source of polyphenols and is been reported as having high antioxidant activity than teas and red wines. In hemolytic anemia, erythrocytes have a shortened life-span which is a part of the clinical syndrome associated with intoxwatayoication, where chemicals interact with sulfhydryl groups, inhibit enzymes, immune mechanisms, and the fragmentation of erythrocytes through the spleen or by unknown or poorly defined mechanisms. This study investigated the potential antioxidant effects of aqueous extract of *Theobroma cacao L.* stem bark on normal and phenylhydrazine (PHZ)-induced anaemic wistar rats. PHZ was used to induce anaemia intraperitoneally at a dosage of 60mg/kg (body weight) for two days. Forty five albino wistar rats weighing 126-224g were grouped randomly into 8 groups of 5 rats each. Group 1 served as normal control, received only water and feed, Group 2 (Anaemic control) was induced with anaemia without treatment, Group 3, 4, and 5 were induced with anaemia, received, 200mg/kg, 500mg/kg and 800mg/kg b.wt of the aqueous extract of *Theobroma cacao* stem bark respectively., while, groups 6, 7 and 8 were normal rats given 1000mg/kg, 3000mg/kg and 5000mg/kg b.wt of the extract for 28days. Some haematological parameters, enzymatic and non- enzymatic antioxidant activities were determined. Results obtained showed that high doses of the extract showed significant increase ($p<0.001$) in SOD activities in the normal rats,Catalase & MDA increased both groups. However, SOD levels reduced significantly in the treated anaemic rats. The increase in some of the antioxidant enzyme activities and concomitant reduction in severity of anaemia in the treated rats points to ameneorative effect of the antioxidant properties of Theobroma cacao L. on drug induce anaemia in wistar rats.

EVALUATION OF *OGOGORO* -INDUCED TESTICULAR TOXICITY IN ADULT MALE WISTAR RATS.

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Excessive alcohol consumption in beverages implicates several disease entities globally. The aim of this study is to evaluate the *Ogogoro* induced testicular toxicity in adult male wistar rats. Twenty (20) rats were divided into four groups of 5 rats (n=5) each. Group I: normal saline 2ml/kg, Groups II-IV: *Ogogoro* 3.5ml/kg, 7ml/kg, 14ml/kg respectively for sixty days. The result showed a significant decrease in serum testosterone and luteinizing hormone (LH) in groups III and IV respectively, when compared to the control. There was a significant decrease in sperm motility, increased number of dead spermatozoa in group IV when compared to the control ($P< 0.05$). Testicular weight and gonadosomatic index were significantly reduced in group IV treated rats. A significant increase ($P< 0.05$), in testicular MDA level in groups III and IV was found when compared to the control indicating increased lipid peroxidation. The testicular homogenate SOD, GPX and CAT activities in groups III and IV were significantly decreased when compared to the control. Ogogoro administered group IV showed irregular seminiferous tubules with epithelial sloughing. Ogogoro cause testicular toxicity via lipid peroxidation which could adversely affect male reproduction.

ASSESSMENT OF LIVER ENZYMES LEVELS IN TYPE 2 DIABETIC RABBITS TREATED WITH COMBINED SUPPLEMENTATION OF SYZYGIUM AROMATICUM (CLOVE) AND FERMENTED ZINGIBER OFFICINALE (GINGER) SUPPLEMENTS

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Monitoring vital organs functional status such as liver may leads to early detection of complications or adverse effects

of therapeutics. The aim was to evaluate the effects of clove and fermented ginger rhizome supplements on liver enzymes concentration in high fat diet induced type 2 diabetes in rabbits. High fat diet (SAF = 69% + Cholesterol = 1% + Ground nut meal = 20% + ground nut oil = 10%) was fed to rabbits for eleven weeks to ascertain diabetic animal model (DAM), thereafter, DAM were treated with supplements for six weeks. Twenty (20) male rabbits (5 weeks of age) divided into four groups (n=5) were used; Group I (Normal control) was treated with standard animal feed (SAF).Group II-IV (DAM groups) were treated as follows: Group II; treated with SAF only, Group III; treated with SAF + cholestran (0.26 g/kg) and Group IV; treated with SAF + clove + fermented ginger supplements, At the completion of treatments, animals were sacrifice and serum from blood samples was used for laboratory assessments of liver enzymes, data obtained were statistically analyzed using SPSS version 20.0. A significant ($P < 0.05$) increase in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in group II (diabetic animals fed on normal feed) were noticed, when compared to normal control group. While in group IV a significant ($P < 0.05$) decrease in serum AST when compared to diabetic rabbits group on normal feed was observed. In conclusion, combined clove and fermented ginger supplements reverses (down-regulates) elevated liver enzymes seen in high fat diet induced type 2 diabetic rabbits. Further work to validate these effects could facilitate the use of the supplement as a composite in formulating diet for type 2 diabetic patients.

EFFECT OF NICOTINAMIDE ON DEPRESSED MICE USING OPEN SPACE FORCED SWIM TEST MODEL OF DEPRESSION.

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Depression is a significant contributor to global burden of diseases and affects people of all communities across the world. The world health organization has ranked depression as the 4th leading cause of disability in the world and is projected to be the 2nd by the year 2020. In Nigeria, about 3.9% of the populations suffer from depression. Nicotinamide (NAM), has an anti-inflammatory property and can inhibit cytokines release, thus, having antidepressant potential. The aim of this study was to investigate the antidepressant effect of nicotinamide in depressed mice using open space forced swim test model of depression. Twenty-five mice of both sexes were randomly divided into five groups of five animals each; group 1 received normal saline 10 ml/kg, group II, III and IV received 25 mg/kg, 50mg/kg and100mg/kg of NAM respectively and group V received 20 mg/kg of fluoxetine. Tail suspension test (TST) was carried out on the animals before treatment which served as a baseline for depression. The animals were habituated to swimming for 4 days and on the fifth day, drug administration commenced which lasted for two weeks. The animals were only allowed to swim on the 1st, 4th, 7th, 10th and 14th day. Behavioural despair (immobility time) and locomotor activity (line crossing) of animals were assessed using tail suspension test, open space force swim test (OSFST) and open field test (OFT) respectively. The result obtained showed no significance. However, a statistically significant difference ($p < 0.05$) in 4th day between group two and five and also on the 7th day between group five when compared with group one and group two. There was no statistically significant difference in immobility of the Tail Suspension Test ($p > 0.05$) and line crossing in Open Field Test ($p > 0.05$) between the control and NAM treated groups. In conclusion, the result showed that NAM has no effect on the immobility time in depressed mice using OSFST.

MODULATORY ROLE OF VITAMINS A AND E ON MEMORY AND MOTOR FUNCTIONS OF CYANIDE INDUCED NEUROTOXICITY IN ADULT SWISS MICE

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Cyanide is a potent neurotoxic substance that can initiate series of intracellular reactions leading to oxidative stress. Vitamins A and E are antioxidants that have scavenging properties against free radicals and reactive oxygen species. This study was designed to evaluate effect of sublethal administration of potassium cyanide (KCN) and possible ameliorative role of vitamins A and E on sensorimotor functions and long term visuo-spatial learning and memory in adult Swiss mice. Thirty-five mice weighing between 18-22 g were used for the study. The animals were randomly divided into five groups (n = 7) and exposed to sublethal concentration of potassium cyanide (10% LD50; 1.5 mg/kg). KCN was administered orally while vitamin A (25 mg/kg) and vitamin E (50 mg/kg) were administered intra-peritoneally (IP) once daily for 28 days. KCN was administered first, followed after 10 minutes by vitamin A, and then vitamin E after 5 minutes. At the end of 28 days, mice were examined for signs of neuro-toxicity using wire grid, coat hanger and stationary beam test models. in the wire grid test, the latency to fall in weeks 2 and 4 were statistically significant ($p < 0.05$). In acquisition and retention, using elevated plus maze (EPM), KCN treated group recorded high transfer latencies in seconds (50.40±1.72 secs) and (57.60±0.93 secs) as compared to group IV (29.40±0.68 secs; 5.60±0.60 secs). It was concluded that KCN affects motor coordination and memory in mice, while treatment with antioxidant vitamins A and E ameliorated these deficits.

AMELIORATIVE EFFECT OF EUGENOL ON ALUMINIUM CHLORIDE-INDUCED NEPHROTOXICITY IN WISTAR RATS

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Kidneys are essential to the urinary system and play critical role in homeostasis. Eugenol is known for its medicinal value and is an established antioxidant present in plants like, clove, basil and nutmeg. This study histologically and biochemically assessed the ameliorative effect of Eugenol on aluminium chloride ($AlCl_3$)-induced nephrototoxicity in Wistar rats. Thirty Wistar rats of bith sexes (95 - 110 g) were divided into six groups (A – F) of five rats each. Group A served as the control and was administered distilled water (2 ml/kg), Group B was administered $AlCl_3$ (100 mg/kg) only. Groups C - F were administered Eugenol (150 mg/kg, 225 mg/kg and 300 mg/kg, respectively), and Silymarin (100mg/kg), before $AlCl_3$ (100 mg/kg). All the Administrations were via oral Gavage for the duration of three weeks. The Ameliorative effect of Eugenol was assessed using light microscopic examination of routinely (H and E) stained kidney sections and biochemical analysis of kidney electrolytes (Na^+, K^+, Cl^- and HCO_3^-) and Urea. Results revealed the distortion of the histoarchitecture of the renal parenchyma and elevated levels of kidney electrolytes in $AlCl_3$-treated group when compared to the control ($p>0.05$) and Eugenol-treated ($p<0.05$) groups. However, administration of Eugenol ameliorated $AlCl_3$-induced kidney damage by preservation of the kidney histoarchitecture and, decreased ($p<0.05$) serum kidney electrolytes levels. Eugenol ameliorative activity was comparable with that of Silymarin, especially at dose 225 mg/kg. Eugenol possesses nephroprotective potentials against heavy metal-induced acute nephrototoxicity in Wistar rats.

WAIST TO HEIGHT RATIO CORRELATES NEGETIVELY WITH DURATION OF MENSTRUAL BLEEDING AMONG FEMALE STUDENTS OF BAYERO UNIVERSITY, KANO.

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Obesity is associated with menstrual irregularity, sub fertility and infertility presumably due to obesity-associated increases in peripheral estrogen production by the adipose tissue. It has been suggested that accumulation of fat centrally may be more associated with menstrual irregularities than overall adiposity such as BMI. This study therefore aimed at assessing the relationship between indices of obesity and menstrual cycle characteristics among female students of Bayero University, Kano. Using self-administered questionnaire the menstrual characteristic of 283 students between the ages 16 to 26 years, were studied. Body mass index (BMI) was calculated as the subject's weight (kg) divided by the square of the subject's height (m^2) and waist-height ratio (WHtR) was calculated as waist circumference divided by height. The data was analyzed using IBM SPSS Statistics for Windows, version 22.0. Quantitative data was presented as mean ± SD, and qualitative data was presented using percentages and frequency. $p\leq0.05$ was considered statistically significant. Most of the respondents 184 (65%) had their weight within normal, ther mean age at menarche was 13.55±1.50; mean length of menstrual cycle was 26.68±3.51; and the mean duration of menstrual bleeding was 5.18±1.24. A statistically significant difference between the body mass index and duration of menstrual bleeding was found. The study also found a significant negative correlation between waist to height ratio (WHtR) and duration of menstrual bleeding.

PROTECTIVE EFFECT OF L-ARGININE CO-ADMINISTRATION WITH HIGH-FAT DIET ON ERYTHROCYTE OSMOTIC FRAGILITY AND MALONDIALDEHYDE CONCENTRATION IN MALE WISTAR RATS

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The excessive consumption of high-fat diet is associated with an increased incidence of obesity, resulting in oxidative stress and lipid peroxidation. This study was designed to evaluate the protective effect of L-arginine co-administration with high-fat diet on erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration in male Wistar rats. Thirty (30) adult Wistar rats used for the study were divided into six groups of five rats each: Group I: (Normal Control) Received distilled water (1 ml/kg) with normal feed. Group II: (Diabetic control) Received high-fat diet (HFD) only. Group III: Received NFD + 200 mg/kg of L-arginine. Group IV: Received NFD + 400 mg/kg of L-arginine. Group V: Received HFD + 200 mg/kg L-arginine. Group VI: Received HFD + 400 mg/kg of L-arginine. The result shows significant decrease ($P < 0.05$) EOF only in the 400 mg/kg Arg+HFD group (84.0±2.28%) (55.4±2.0%) as compared to normal control (90.6±1.72%) (60.4±2.02%) and HFD-only group (92.4±1.60%) (62.8±0.92%) at 0.3% and 0.4% respectively. The result on MDA concentration showed a non-significant increase ($P > 0.05$) in all the treated groups when compared to normal group. The highest MDA concentration was observed in the group treated with high-fat diet only (0.96 ± 0.10 µMol/L), when compared to normal (0.72 ± 0.10 µMol/L). It was concluded that HFD increased haemolysis and MDA concentration in Wistar rat, and this effect was ameliorated by L-Arginine administration.

EVALUATION OF THE AQUEOUS EXTRACT OF *Datura stramonium* ON OXIDATIVE STRESS BIOMARKERS OF *Plasmodium berghei*-INFECTED MICE

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Malaria is one of the major infectious diseases in Africa, especially in Nigeria. The aim of this study was to evaluate the effect of an aqueous extract of *Datura stramonium* on some oxidative stress biomarkers of *Plasmodium berghei*-infected mice. The aqueous extract of the leaves at 250, 500 and 1000 mg/kg body weight/day dose levels were used to treat the test groups after infection for four days (Adia *et al.*, 2014), while a standard antimalarial drug, Cloroquine, at a dose of 25 mg/kg body weight was administered on the positive control group. The negative control group was left untreated. The variation in the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) in the different groups were observed throughout the study. The crude extract was screened for its phytochemical composition. Results showed a marked decrease in GSH level and activity of SOD and GSH after infection signifying oxidative stress. However, there was a significant rise (P<0.05) in SOD and GSH levels in the group treated with 500 mg//kg body weight of *D. stramonium* as compared to other treatment groups. More so, the activity of catalase across groups treated with *D. stramonium* also showed considerable increase. The screening for the phytochemical composition of the crude extract showed the presence of alkaloids, flavonoids, and saponins, while tannins and anthraquinones were absent. The findings of this study showed that *Datura stramonium* may be used as an antimalarial regimen, as its' application does alleviate oxidative stress as seen in the biomarkers determined.

ANTIDIARRHOEAL ACTIVITY OF METHANOL LEAF EXTRACT OF *HYPTIS SUAVEOLENS* (L.) POIT (BUSH MINT).

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Diarrhoeal disease is prevalent in tropical countries with poor hygiene and low standard of living. Diarrhoea is a leading cause of malnutrition and death due to its dehydrating effect and loss of electrolytes. *Hyptis suaveolens* has been used traditionally around the world for various ailments and diseases. The plant is also consumed as foods in some communities. The phytochemical analysis and acute toxicity test of the methanol leaf extract were evaluated. Antidiarrhoeal activity of the leaf extract was evaluated using castor oil and magnesium sulphate induced diarrhoea in rats and chicks at doses of 250 mg/kg, 350 mg/kg and 500 mg/kg body weight orally. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins and triterpenes. The oral LD_{50} of the extract was above 5000 mg/kg in both rats and chicks. The methanol leaf extract of *Hyptis suaveolens* at tested doses significantly (p<0.05) reduced the frequency and weight of stool in castor oil and magnesium sulphate - induced diarrhoea in rats and chicks in a dose and time dependent manner compared to loperamide (2 mg/ml). It can be concluded that *Hyptis suaveolens* has antidiarrhoeal activity in rats and chicks which may be attributed to some of the phytochemical constituents.

POLYUNSATURATED FATTY ACID METABOLISM AND ANTIOXIDANT RESPONSES IN ADULT LACTATING WISTAR RATS FOLLOWING ORAL ADMINISTRATION OF MONOSODIUM L-GLUTAMATE (MSG)

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Several studies have reported toxic effects of MSG on the reproductive system however; the concept of oxidative shielding suggests that during reproductive exercises the likes of lactation, mothers preemptively decrease their levels of oxidative stress for the sole of purpose of shielding or protecting their offspring from detrimental effects of damaged molecules like oxidized fatty acids. Hence this study was designed to investigate the effect of MSG on oxidative status of Wistar rats during lactation. Following birth, the dams were divided into four groups of six (n=6) rats each having six pups. Oral administration of MSG lasted for 2 weeks as follows: Group 1: Distilled water (2 ml/kg), Group 2: Metoclopramide (5 mg/kg), Group 3: MSG 1850 mg/kg and Group 4: MSG 3700 mg/kg. The result of serum malondialdehyde (MDA) concentration (Umol/L) was lower significantly (P< 0.05) in all the MSG administered groups compared to control and metoclopramide (5 mg/kg) treated. Serum superoxide dismutase (SOD) (Umol/mg) was significantly higher (P< 0.05) in MSG administered groups compared to metoclopramide (5 mg/kg). Serum level of glutathione (GSH) (Umol/mg protein) was significantly higher (P< 0.05) in MSG 3700 mg/kg administered group compared to control, MSG 1850 mg/kg and metoclopramide (5 mg/kg). Although serum catalase (CAT) level was decreased in both MSG and metoclopramide groups compared to control, the difference was however only statistically significant (P< 0.05) in 3700 mg/kg group. Oral administration of MSG increased serum SOD and GSH level with a significant decrease in MDA concentration in lactating Wistar rats.

INFLUENCE OF VARYING DEGREE OF WOOD DUST EXPOSURE ON PULMONARY FUNCTION AND RESPIRATORY SYMPTOMS AMONG WOOD WORKERS IN KANO, NORTH WESTERN NIGERIA.

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One of the major occupation-related health challenges encountered by wood workers is respiratory disorder, which usually results from breathing in noxious or toxic chemicals such as wood dust. The aim of this study is to

evaluate the respiratory functions and symptoms among wood workers exposed to varying degrees of wood dust in Kano, Nigeria. This descriptive cross-sectional study was carried out among 370 randomly selected wood workers in Kano wood market. Lung function test was performed, while semi-structured interviewer administered questionnaire was used to rate respiratory symptoms. The study demonstrated that there is low percentage predicted force expiratory volume at one minute ($PPFEV_1$) and percentage predicted ratio of FEV1 and FVC, whereas, the percentage predicted forced vital capacity (PPFVC) of the respondents across all age groups remained unchanged. A statistically significant association existed between exposure to wood dust and respiratory symptoms (χ_2 = 16.2, df = 1, p = 0.001), thereby contributing to the observed manifestation of respiratory symptoms such as chronic cough, corrhiza, breathlessness and wheezing among 61% of wood dust exposed workers. Similarly, a negative correlation was observed between degree of exposure to the hazards and lung function of the workers (r = -0.655, P-Value = 0.0001).

COMPARATIVE EFFECT OF *Nigella sativa* SEED OIL AND ZINC GLUCONATE ON ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE AND GASTRIC SECRETION IN WISTAR RATS

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A wide spread search has been launched to identify new anti-ulcer therapies from natural Sources, with minimal side effects. The aim of the study was to compare the effects of *Nigella sativa* seed oil and zinc gluconate on ethanol-induced gastric mucosal damage and Secretion in Wistar rats. A total of 50 male rats, weighing between 150-200g were used for the study. The animals were then sub-divided into two sub-groups of 25 rats each for gastric mucosal damage and gastric secretion studies respectively. Each of the sub-groups were divided into 5 groups of 5 rats each and treated with distilled water (10ml/kg), absolute ethanol (1ml/kg), *Nigella sativa oil* (5ml/kg), zinc gluconate (50mg/kg) and zinc gluconate (50mg/kg) plus *Nigella sativa* oil (5ml/kg) respectively. The results of the study revealed the normal architecture of gastric mucosa for the control group with ulcer index of 0.0 ± 0.0 mm. The ethanol-treated group showed severe necrosis-of gastric epithelium, with ulcer index of 10.25 ± 0.85 mm as compared to the control. The *Nigella sativa* oil, zinc gluconate and *Nigella sativa* plus zinc gluconate treated groups have demonstrated significant decrease in the ulcer indices of 5.50 ± 1.04 mm, 4.75 ± 0.25mm and 3.75 ± 1.44mm respectively as compared to the control with 0.0 ± 0.0 mm. On the other hand the groups also showed preventive indices of 48.8%, 53.6% and 63.4% respectively when compared to those of the control 100% and ethanol treated group with no protection. In conclusion, *Nigella sativa* and zinc gluconate conferred protection to gastric mucosa but the combination of the two produced an additive effect.

LAURIC ACID ALLEVIATES INFLAMMATION AND STRUCTURAL CHANGES IN THE LUNGS OF TYPE II DIABETIC MALE WISTAR RATS

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Diabetic complication in the lungs is characterized by infiltration of inflammatory mediators in the lungs and structural alteration of the lung parenchyma. This study was designed to evaluate the effect of lauric acid on leucocytes infiltration in **bronchoalveolar lavage fluid** (BALF), concentration of TNF-α and lung histology of type II diabetic male Wistar rats. Type II diabetes was induced using high fat diet/40 mg/Kg streptozotocin along with 20% fructose solution. A total of thirty-five male Wistar rats were randomly divided into seven groups of five rats each as follows: Group **I** was normoglycemic Wistar rats administered 1ml/Kg distilled water, and served as normal control. Group II was normoglycemic Wistar rats administered 125 mg/Kg lauric acid. Group III was diabetic Wistar rats administered 1ml/Kg tween 80, and served as diabetic control. Groups IV, V, VI and VII were diabetic Wistar rats treated with 125 mg/Kg, 250 mg/Kg, 500 mg/Kg lauric acid and 100 mg/Kg metformin orally respectively. The results obtained, showed a significant ($P \leq 0.05$) increase in total and differential white blood cell count in blood and BALF of the diabetic rats, however, it was significantly decreased after treatment with lauric acid. The concentration of TNF-α was significantly higher in the lungs of diabetic rats, but treatment with lauric acid has reduced it significantly. Lauric acid also reversed the reduced alveolar spaces in diabetic lungs. It can be concluded that lauric acid reduced inflammation and reversed the histoarchitectural alterations in the lungs of type II diabetic male Wistar rats.

TAURINE TREATMENT MODULATES HEMATOLOGICAL INFLAMMATORY MARKERS IN PAPAIN INDUCED ARTHRITIS IN ADULT FEMALE WISTAR RAT.

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Osteoarthritis (OA) is one of the most prevalent and debilitating joint disease associated with reduced quality of life and increased healthcare costs. Available medical therapies, including traditional analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) for OA are ineffective at slowing down the disease progression, but rather alleviate the symptoms by reducing pain. Additionally, their chronic use has been linked to number of deleterious side effects. This study aimed to evaluate the effect of taurine on some inflammatory hematological parameters in papain induced osteoarthritis in adult female

Wistar rats. Thirty female rats were acclimatized and randomly divided into six (6) groups: control group (G1), disease control group (G2), standard control group (G3), taurine treated groups (G4, G5 and G6). G1 received distilled water 1ml/Kg, G2 received 1ml/Kg of distilled water, G3 received Diclofenac sodium 5mg/Kg, while taurine treated groups G4, G5, and G6 received 100mg/Kg, 200mg/Kg and 400mg/Kg of taurine respectively. OA was induced by intra-articular injection of papain into the right knee joint of the rats on days 1, 4 and 7 before the commencement of the treatment. The parameters checked include; Erythrocyte sedimentation rate (ESR), Neutrophil lymphocyte ratio (NLR), Platelet lymphocyte ratio (PLR) and diameter of inflammation on the knee joint. There was a significant ($P<0.05$) reduction in ESR, NLR, PLR and diameter of inflammation induced in the knee joint in the taurine treated groups as compared to the disease control group. In conclusion taurine was able to decrease inflammatory hematological parameters in papain induced arthritis in female Wistar rats.

PROTECTIVE EFFECT OF CO-ADMINISTRATION OF VITAMINS C AND E ON RESERPINE-INDUCED MOTOR IMPAIRMENT IN MICE

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The conventional treatments for Parkinson's disease, the most common movement disorder worldwide, have not been able to halt its progression, hence, newer approaches targeting its pathogenesis are being explored. We investigated the effect of combining vitamins C and E on reserpine-induced motor impairment in mice. Twenty-five mice were assigned into 5 groups. Group I (control) received distilled water only while the other groups received reserpine 0.1mg/kg intraperitoneally on alternate days. In addition, Group III (vitamin E group) received vitamin E 200 mg/kg/day, group IV (vitamin C group) received vitamin C 250 mg/kg/day and group V (co-administered group) received both vitamins orally. Group II (reserpine group) received nothing in addition to reserpine. All drugs were given concurrently for 28 days. Neurobehavioral assessment was performed using beam walking and open field tests. Results were expressed as mean ± SEM and values at $p < 0.05$ were considered significant. The increase in number of foot slips (3.60 ± 0.68) as well as the time taken to reach the safe platform (36.60 ± 5.78 s) observed in the reserpine group were significantly decreased in the co-administered group (0.25 ± 0.25 and 3.00±0.41s respectively). The transfer latency was significantly decreased (10.33 ± 1.45 s) with a marked increase in the number of lines crossed (56.00 ± 13.53) in the co-administered group compared to reserpine group (214.00 ± 64.16 s and 4.3 ± 1.67 respectively). The co-administration of vitamins C and E confers a significant neuroprotection against reserpine-induced motor impairment in mice.

INVESTIGATION OF THE EFFECTS OF AQUEOUS EXTRACT OF *Piper guineense* (ASHANTI PEPPER) SEED ON INDICES OF HEPATIC FUNCTIONS

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Piper guineense seed is widely consumed in some part of West Africa for its nutritional and medicinal properties. This study investigated the effect of its aqueous extract on indices of hepatic functions. Twenty Wistar rats were purchased and divided into four groups of five per group. They were allowed access to feed and water for two weeks. Different concentrations of the extract were administered to the three experimental groups: 100 mg/Kg, 200mg/Kg, 400 mg/Kg, while control group were given feed and water. The feeding lasted for 21 days. The result showed that liver enzymes Alanine transaminase, aspartate aminotransferase, Alkaline phosphatase showed no significant difference ($p>0.05$) between the experimental groups and control groups. Total protein and Globulin of the experimental groups were significantly ($p<0.05$) lower when compared to the control group but albumin did not. The group that received the highest dose of the extract had significantly lower total bilirubin when compared with the control group ($p<0.05$) while conjugated bilirubin did not. Histology showed that only high dose of *Piper guineense* distorted the normal histo-architecture of the liver. Thus, moderate consumption of *Piper guineense* seeds is recommended; high dose may be harmful to the liver.

EFFECT OF ETHANOL EXTRACT OF *ALLIUM SATIVUM* ON HEMATOLOGICAL PARAMETERS IN E *COLI* INDUCED SEPSIS IN WISTAR RAT

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One of the agents of sepsis include the gram negative E. coli. Over the years of study, spices, such as Allium sativum, seems to combat infection. The aim of this work is to determine the effect of ethanolic extract of allium sativum on hematological parameters and osmotic fragility of red blood cells of Wistar during E. coli induced sepsis. Thrity-five (35) rats were used in this study and were grouped into 7 groups (n=5). Except for group 1 (normal control), all groups were induced with sepsis by E coli interperitoneal injection. Successful induction was confirmed after 5 days. All groups, except group 1 and group 2, the negative control group, were treated for 14 days. The remaining groups were treated with 400mg/kg/day of hydrochloride ciprofloxacin; HC (group 3; positive control), 200mg of extract/kg/day (group 4), 400mg of extract/kg/day (group 5), 200mg of extract /kg/day with HC (group 6) and 400mg of extract/kg/day with HC (group 7). After 14 days, the hematological parameters and RBC osmotic fragility test were obtained and analyzed ($P<0.05$ and $P<0.01$). Though the extract seems to boost the hematocrit and hemoglobin concentration, it also made them more fragile especially in

the presence of Cirpofloxacin. These could lead to hemolytic anemia. Garlic should be used with hematinics to treat infection. Combined therapy of Garlic and standard drug should not also be encouraged as it worsens the anemia caused by standard drug.

ELECTROCARDIOGRAM PATTERN AND BLOOD PRESSURE IN ADULT SICKLE CELL ANAEMIA PATIENTS IN SOKOTO, NIGERIA

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The present study was aimed at determining the electrocardiographic (ECG) pattern of sickle cell anaemia (SCA) in adults attending the sickle cell clinic at the Haematology Outpatient Unit of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. A total of 148 subjects comprising 55 SCA patients (in crisis), 53 SCA patients (in the steady state) and 40 normal control groups were studied. Following ethical approval, ECG and blood pressure (BP) were measured using standard methods[1]. Electrodes were positioned as recommended by the American Heart Association[2] and BP with the aid of a mercury sphygmomanometer. Patients had significantly (P<0.001) lower systolic BP in the steady state (108±1.6mmHg) and in crisis (105±1.7mmHg) compared to controls (122.9±1.7mmHg). The diastolic BP of patients in the steady state (63.0±1.9mmHg) was significantly (P<0.001) lower than in the control group (73.1±1.7mmHg) but did not differ from the value recorded in patients in crisis (65.5±1.6mmHg). There was no significant difference in the heart rate, P wave duration, QTc and PR interval of patients and control. However, QRS duration was significantly (P<0.0001) lower in SCA patients in the steady state (54.2±2.2ms) and in crisis (53.3±2.8ms) than in controls (72.1±3.0ms). In conclusion, this study shows that the SCA patients had lower BP and QRS duration than healthy controls.

DIURNAL FLUCTUATIONS IN MECHANICAL, THERMAL AND CHEMICAL PAIN THRESHOLDS IN MALE AND FEMALE WISTAR RATS DURING HARMATTAN SEASON IN NORTH WESTERN NIGERIA

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This study investigated the diurnal fluctuation of mechanical, thermal, chemical pain in the light phase and dark phase and also considering their sex based differences during the harmattan season conducted in the North-West Nigeria. The work was carried out in two phases (light and dark phase). Light phase done at 07:00h-10:00h, while Dark phase at 19:00h- 22:00h. The rats were made to undergo the experimental pain assessment (Mechanical, thermal and chemical), also, the temperature and the relative humidity of the day were checked and recorded hourly. Animals were randomly grouped in to two phases, the light phase group and the dark phase group. Animals in the light phase group were grouped in to male and female groups. Animals in the male group were further sub grouped in to three groups of 5 animals each for the three different pain threshold assessment test. Mechanical and thermal pain threshold showed no statistically significant difference between the light phase and the dark phase ($p > 0.05$). Chemical pain threshold was significantly higher in the dark phase when compared to the light phase ($p< 0.05$).

IMPAIRED SPERM PARAMETERS IN STREPTOZOCIN INDUCED DIABETIC MALE WISTAR RATS AND THE EFFECTS OF LAURIC ACID

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Diabetes mellitus is associated with a decline in reproductive function. Studies have shown that coconut oil (CO) possesses anti-diabetic properties and ameliorative effects on impaired sperm parameters. Lauric acid (LA) is the most abundant constituent of CO. Thus, this study sought to investigate the effects of LA on sperm parameters in diabetic male Wistar rats. The animals, divided into 6 groups of five (n=5), received treatments orally for 4 weeks as follows: Group I: distilled water (1ml/Kg), Group II: Diabetic untreated, Group III: Diabetic + LA (90 mg/Kg), Group IV: Diabetic + LA (180 mg/Kg), Group V: Diabetic + LA (360 mg/Kg) and Group VI: Diabetic + CO (1.42 ml/Kg). The results show a significant decline ($P < 0.05$) in the concentration, motility, normal morphology and viability of sperm cells in diabetic untreated rats compared to the normal control rats. In diabetic rats treated with CO; sperm concentration, percentages of motile, normal and viable sperm cells were significantly higher ($P < 0.05$) compared to the diabetic untreated rats. Compared to the diabetic untreated rats; only the percentage of progressive motile sperm cells in diabetic rats treated with 90 mg/Kg LA and the percentage of normal sperm cells in diabetic rats treated with 360 mg/Kg LA respectively, were significantly higher ($P < 0.05$). Furthermore, the impacts of the above doses of LA were significantly lower ($P < 0.05$) compared to the treatment with CO. Thus, impaired sperm parameters in diabetic rats were not completely alleviated by lauric acid.

EFFECT OF STEP AEROBICS ON BLOOD GLUCOSE LEVEL AND CARDIORESPIRATORY PARAMETERS OF OVERWEIGHT ADULTS IN VOM, PLATEAU STATE, NIGERIA.

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This study was aimed at determining the effect of step aerobics training on blood glucose and cardiorespiratory parameters of overweight adults in Vom, Plateau State, Nigeria. Twenty (20) overweight adults participants were selected using the purposive sampling technique from Vom, Plateau State, Nigeria. Participants were trained for 8weeks with a frequency of 3 days per week and a duration of 48minutes with a moderate intensity of HR max of between 50-65%. Blood Glucose Level (BGL), Resting Heart Rate (RHR), Peak Expiratory Flow Rate (PEFR) and forced expiratory volume in one second (FEV_1) were taken at pre training and post training(after 8 weeks of step aerobics training) respectively. Results showed that Step aerobics training significantly reduced the BGL of overweight adults ($P<0.05$), caused no reduction on the RHR of overweight adults ($p>0.05$) and increased the PEFR and FEV_1 of overweight adults ($P<0.05$). The effect of step aerobics on the overweight adults has proven to be generally positive on the basis of these findings, therefore Step aerobics should be publicized in fitness and wellness centers as a mode of training as it has shown evidence of metabolic and cardiorespiratory adaptations in overweight adults by causing a reduction in blood glucose level, increasing peak expiratory flow rate and forced expiratory volume in one second.

EFFECT OF LACTATIONAL EXPOSURE TO FLAVONOID-RICH EXTRACT OF *HIBISCUS SABDARIFFA* ON THE ONSET OF PUBERTY AND REPRODUCTIVE HORMONE PROFILE IN THE OFFSPRING OF ALBINO RATS

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The effect of lactational exposure to flavonoid-rich extract of *Hibiscus sabdariffa* from birth to postnatal (PND) 42 was investigated in Albino rats. Sixteen in-bred pregnant rats were randomly assigned to four groups (A, B, C, D) of four rats each and were given rat chow and water adlibitum. From birth to PND 21 (weaning), all groups had normal rat chow and water adlibitum. The dams of the test groups were administered with rich extract of flavonoid via oral gavage daily, 10mg/kg body weight (B), 20mg/kg body weight (C) and 50mg/kg body weight (D), throughout the entire period of lactation (PND 0–– PND 21). At PND 21, the pups were designated into four groups according to their dams. Water and fluid intake was measured daily. From PND 21 pups were monitored daily for Balano-preputial separation and Vaginal opening. At PND 42, blood sample was collected by occulo-puncture for the assay of reproductive hormones of both male and female pups. Results from this study showed a significant ($P>0.05$) delay on the onset of puberty, increase in weight and Body Mass Index (BMI) of the test rats as compared with the control. Also, there was a significant ($P>0.05$) decrease in circulating plasma levels of LH, FSH and Testosterone (male), while there was a significant increase ($P<0.05$) in the circulating plasma level of Estradiol (female) of these pups. Flavonoid was seen to delay the onset of puberty and decreased circulating levels of reproductive hormones.