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Aim/Scope

Bayero Journal of Biomedical Sciences (BJBS) is a journal that publishes original research and critical reviews in the fields of Anatomy, Biochemistry, Microbiology, Nutrition and Dietetics, Pharmacology and Physiology. It is a biannual publication (June & December) with intent to publish quarterly in the future depending on availability of accepted manuscripts.

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Articles for publication should be sent to the Editor-in-Chief, BJBS, Faculty of Basic Medical Sciences, Bayero University, PMB. 3011, Kano or through the Journal email address: bjbs.bms@buk.edu.ng

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Text: The text of the manuscript should appear in the following order: Title, Authors name(s) and address(es), Abstract, Keywords, Introduction, Materials and Methods, Results, tables, figures, and plates, Discussions, Conclusion, Acknowledgement (if any), References (authors should please comply strictly to this format).

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Acknowledgement: Optional and should acknowledge support and assistance rendered by individual(s) or organisations.

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- ❖ Recent research ^[1] reported the value of DNA markers in this respect.
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❖ The analysis was carried out according to the method reported by Muhammad *et al.* ^[12]

References should be numbered consecutively in the order in which they are first mentioned in the text. In the sequentially numbered reference list at the end of the manuscript, the titles of journals should be given in full. However, abbreviated journal names are acceptable for indexed journals according to the style used in Index Medicus. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source. Avoid citing a "personal communication" unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text.

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4. Web page/website: World Health Organization. Global diabetes compact. Available from: www.who.int/news/global-diabetes-compact (accessed 12th May, 2020).

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Bayero Journal of Biomedical Sciences (BJBS) *(Bayero J. Biomed. Sci.)*

Editorial

The Editorial Board of Bayero Journal of Biomedical Sciences (Bayero J. Biomed. Sci.), Bayero University, Kano is proud to present its **volume 6, issue 1** to the teeming scholars and young scientists across the globe, who like to be abreast with current research findings in the fields of Biomedical Sciences. The Journal is an official publication of the Faculty of Basic Medical Sciences, Bayero University, Kano, Nigeria. Due to so many logistic problems, the biannual publication has not been archived for the past four years. We are only able to print once a year, but we are hoping to improve in due course. The journal contains well – researched scientific works that cover six different areas; Anatomy, Biochemistry, Microbiology, Nutrition and Dietetics, Pharmacology and Physiology.

The Publication of the journal is to ensure wide dissemination of scientific research findings that will ginger the academic community to promote human development using evidence – based findings and also to create synergy and collaboration in the areas within the scope of the journal. This is no doubt, a noble idea that will advance knowledge in these areas. We encourage scientists to focus their researches more on recent happenings in the field of medical sciences especially on the issues of aftermath of Covid-19 global pandemic, effects of Covid-19 vaccine and its potential side effects, climate change, pollution, industrialization, displaced and vulnerable communities, so as to bring out new evidence based information in these areas. We will ensure that expected standard is maintained in all the subsequent editions with the help of our highly qualified team of experienced reviewers.

Scientists in the academic environment, research institutes and industries are therefore encouraged to actively participate in disseminating their works through this medium. It is pertinent to thank our team of talented professionals who ensured promptness in the editing processes of the manuscripts sent to them despite their tight schedules. This will encourage patronage of the journal and help in advancing the career of the contributing authors. The Editorial Board members deserve commendation for being resolute in promoting the journal. Thank you for the support, cooperation and encouragement.

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Memory impairment in Prenatally Exposed Rat (*Rattus norvegicus*) to Hyoscyamine Fraction of *Datura stramonium* L. Seeds

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Abstract

The study investigates the effects of prenatal exposure to hyoscyamine fraction of (*Datura stramonium*) *D. stramonium* seeds on memory in adult Wistar rats. Fresh seeds of *D. stramonium* were macerated with ethanol, hyoscyamine was partition-fractionated with chloroform and quantified using high-performance liquid chromatography (HPLC). Sixteen (16) Wistar rats with an average weight of 250±0.05 grams comprised of equal gender were used for the study. The rats were mated and divided into control and treated groups. Equivalent body weight of normal saline and 200 mg/kgbw of hyoscyamine fraction were orally administered respectively on gestational days (GD) 15-20. The litters obtained were nursed to maturity. On PND 60-75, the rats were tested for memory impairment using Morris water maze (MWM) for spatial memory and Novel object recognition test (NORT) for short term memory. Data were expressed as mean ± SEM. Student's *t*-test and repeated measures ANOVA with Fisher's multiple comparisons post-hoc tests were used to compare the mean differences using Minitab 17 (LLC., U.K.) Values with $p < 0.05$ were considered statistically significant. There was a decrease in the meantime taken to explore the novel object which was statistically significant at ($p = 0.012$). There was also a decrease in the meantime to locate the escape platform which was statistically significant at ($p = 0.001$). There was a mild neurodegeneration with cytoplasmic vacuolations in the CA1 hippocampal region of the treated group. In conclusion, prenatal exposure to hyoscyamine fraction of *D. stramonium* impaired both short term and spatial memories retention ability in the hippocampus of adult Wistar rats.

Keywords: Hippocampus, hyoscyamine, prenatal, memory, neurodegeneration

Introduction

Medicinal plants play a significant role in traditional medicine during pregnancy, childbirth and postpartum care. Other usage includes female fertility, menorrhoea, birth control, pregnancy, birth, puerperium and lactation [1-6]. Abuse of certain plants can produce a wide range of clinical effects such as psychotic, stimulant, sedative, euphoric, and anticholinergic symptoms [7]. Women are the primary consumers of herbal medicines (HMs) and usually continue to use during pregnancy [8]. Pregnant women usually perceive herbal products as

a safe, natural alternative to conventional drugs [9] and often use them to improve their wellbeing or for the treatment of non-life-threatening conditions (e.g., nausea, constipation). According to a 2010 survey conducted by the National Institute on Drug Abuse (NIDA), 16% of pregnant women aged 15 to 17 years, 7.4% of pregnant women aged 18 to 25 years, and 1.9% of pregnant women aged 26 to 44 years abuse illicit substances [10/11]. From 2002 through 2010, the rate of reported illicit drug use among pregnant women aged 15 to 44 years rose from 3%

to 4.4% [10, 12]. The maternal abuse of narcotics has also risen because of “more liberal use of prescription opiates in pregnant women to palliate acute and/or chronic pain [13].” Several authors have described potential adverse effects arising from the use of medicinal plants in pregnancy, such as teratogenic, mutagenic, embryotoxic and abortifacient effects [14-18].

Despite the use of *D. stramonium* for thousands of years [19], a good number of pieces of literature have cautioned against the use of it during pregnancy [20]. *D. stramonium* was used traditionally to treat asthma, gastrointestinal problems, aches, abscesses, arthritis, boils, headaches, abortifacient, rattlesnake bites, sprains, swellings, and tumours. It was also used for relieving the pains of rheumatism and sciatica as an ointment and easing spasms of Parkinson’s disease [21]. Ayurvedic medicine, the plant was used for the treatment of ulcers, wounds, inflammation, sciatica, bruises and swellings, rheumatism, gout, asthma bronchitis and toothache [22]. In Nigeria, the juice of the leaves mixed with warm milk was used to expel intestinal worms including cestodes, whereas the seeds mixed with palm oils used externally for insect bites and stings [23-27].

The abortifacient and teratogenic properties of ethanolic extract of *D. metel* leaf were established [28]. Although pieces of literature have shown that hyoscyamine act as anti-muscarinic compounds both on the central nervous system (CNS) and peripheral nervous system [29], records on the effects of hyoscyamine fraction of *D. stramonium* on cognitive and spatial learning memories in adulthood following prenatal exposure was scanty. The present study, therefore, investigated the effects of prenatal exposure to hyoscyamine fraction of *D. stramonium* seeds on the memory in the hippocampus of Wistar rats.

Materials And Methods

Plant Materials, Extraction and Fractionation

A fresh *D. stramonium* seed was procured from Sharada residential area of Kano Municipal Local Government, Kano State, Nigeria. The seeds were identified and a voucher number (VN108) was issued at the herbarium of the Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The seeds were

extracted and partition-fractioned according to Kamada *et al* [30] and Djilani *et al* [31]. Two thousand grams of the dried seeds were weighed using a digital weighing machine, grounded to a pulp using an electronic blender. The pulverized seeds powder was cold macerated with 70% ethanol and partition-fractioned with A 5 ml portion of 10% sulphuric acid (H₂SO₄). The quantity of the fraction was obtained by the high-performance liquid chromatography (HPLC). Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

Experimental Animals

Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2018/042). Eight (8) apparently healthy Wistar rats comprised of equal numbers of adult males and virgin females were procured from the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, Bayero University, Nigeria. The animals were transported by road to the Animal House of the Pharmacology Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria (ABU), Nigeria. The males were separated from the females, housed and allowed to acclimatize for two weeks at ambient temperature, with alternate day and night cycles natural condition. Rat chow (*Vital feeds*®) and water were made available to the animal’s *ad libitum*. Animals handling and procedures were according to Ahmadu Bello University Zaria, Nigeria. The median lethal dose (LD₅₀) of hyoscyamine fraction was determined using Lorke’s method [31].

Experimental Design

A total number of sixteen (16) adult Wistar rats comprised of equal gender were used. The female rats were injected with an equivalent bodyweight of Zoladex® (3.6 mg AstraZeneca) intraperitoneally to synchronize their oestrus cycle. Twenty-four hours after the synchronization, vaginal smears were collected by vaginal lavage [32] using a 1 ml plastic pipette filled with 10 µL of normal saline (NaCl 0.9%). A different glass slide was used for each rat, and unstained material was viewed under a light microscope, without the use of the condenser lens, at 10 x objective lenses [33]. Round and nucleated cells are epithelial cells; irregular epithelial cells without a nucleus are the cornified cells, and the little round

epithelial cells are the leukocytes were recognized (plate I). The proportion among them was used for the determination of the oestrous cycle phases [33]. The animals were randomly selected and divided into two (2) groups; control and treatment. Each group contained a total of eight (8) rats in the ratio of 1:1 adult male to virgin female. Animals in each group were allowed to mate freely and evidence of mating was established by the presence of sperm tails in the vaginal smears collected and viewed

under a light microscope after 24 hrs (see plate IIb). The pregnant dams detected, were isolated and transferred to maternity cages separately in different cages. The control and treated groups received an equivalent bodyweight dose of normal saline and 200 mg/kgbw of hyoscyamine fraction of *D. stramonium* seeds respectively orally from gestational day (GD) 15 – 20. The pups delivered were nursed to adulthood under natural condition.

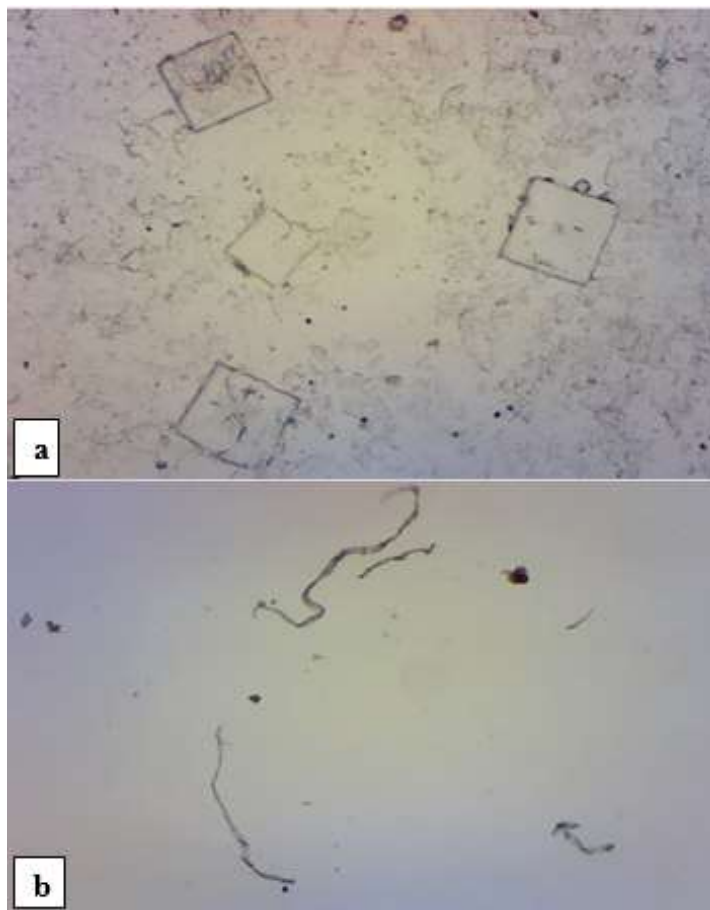


Plate Ia&b: Proestrous phase and sperm tails in vaginal smears of Wistar rats showing nucleated leukocytes and sperm tails 24 hrs after mating (x 10 magnification).

Neurobehavioral Test

Novel Object Recognition Test

The aim of this was to test short-term memory [34] after administration of the hyoscyamine fraction of the *D. stramonium* seeds. The test consisted of three phases i.e. (habituation, sampling and test) which was completed in two days. For each phase of this test, the open field arena was thoroughly cleaned

with an unscented bleach germicidal wipe, 70% Ethanol followed by distilled water before initial use. A day before object exposure, the rats were habituated to the open field arena in a 50 x 50 cm wooden box. Before habituation session, a SONY® (Model DCR – PJ5E) digital and a video camera was used for a proper video covering of the rats in the maze. A rat at a time was gently removed from the

home cage and placed in the centre of the arena. The video covering system was turned on and the rat was allowed to freely explore the arena for 10 minutes. At the end of every session, the arena thoroughly sanitized before the next session began. This was repeated for all the rats until all got habituated the arena. The same protocol was observed during the sampling and test phases only that, two identical objects (A_1 and A_2) were used and two unidentical objects (A and B) objects for were used 15 minutes respectively.

Morris Water Maze

The aim was to test spatial learning and memory [35] after administration of the hyoscyamine fraction of the *D. stramonium* seeds. This was carried out for six consecutive days using a modified Morris method [36]. The apparatus consisted of a circular Aluminium tank of 100 cm diameter and 60 cm depth with an escape platform of 20 cm long and 12 cm diameter, filled with a pool of clean water of about two-third of the tank at 22 – 25°C, deep enough to expose 2.54 cm (1 inch) of the platform above the water surface. A digital video device, SONY® (Model DCR – PJ5E) was suspended directly over the pool to capture the entire setup. The rats were trained for 5 days with methylene-blue coloured water that submerged the platform about 1 inch beneath. A latency period of 60 sec was allowed for each rat to find the platform. This was repeated for all the rats at five different locations by changing the positions of the platform in the pool within the N, E, S, and W directions following Qing *et al* [37] protocol. The same protocol was observed during the test day 6, however, 30 seconds for trial and the escape platform was removed. The time taken for each rat to identify the usual position of the platform was recorded and while all videos recorded for the trials were analyzed for the escape latency. The object bias score was calculated according [38].

Animal Sacrifice and Histological Methodology

The animals were euthanized using 75% Ketamine (10 mg/ml USP) anaesthesia, the brains were dissected, removed and preserved in Bouin's fluid for histological procedures. The tissues were processed in the Department of Pathology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, Kaduna state, Nigeria. The brain tissues were dehydrated in different grades of alcohol and cleared in xylene using an automatic

processing machine (Shandon Southern Duplex Processor). The tissues were then infiltrated with paraffin wax and blocked in the coronal plane. Serial sections of the blocks were taken at 8 μ m with a (Leitz Wetzlar) microtome, mounted on glass slides and allowed to dry overnight. The staining technique employed was hematoxylin and eosin in paraffin sections [39]. Sections were then observed under a light Olympus Binocular Microscope (Ch-20i, Uttar Pradesh, India) high magnifications ($\times 40$) and micrographs were taken with the help of Celestron® eyepiece digital camera (EC 3.0 MP, China). Coronal sections of the hippocampus were observed in the treated rats and compared to the controls.

Statistical Analyses

Data obtained were expressed as mean $\pm$ SEM. Student's *t*-test was carried out for the novel object recognition test. Repeated measures ANOVA followed with Fisher's multiple comparisons post-hoc was carried out to find the mean differences in the escape latency, exploration, discrimination and novelty preference time between and within groups using Minitab 17 (LLC., U.K.) statistical package software. $P < 0.05$ were considered statistically significant. All figures and charts were constructed using GraphPad Prism 8.

Results

Figure 1 shows comparisons of the spatial learning and memory test between the control and prenatally treated, 200 mg/kgbw hyoscyamine fraction Wistar rats using repeated-measures ANOVA followed by Fisher's pairwise post-hoc test. There was a decrease in the meantime to locate the escape platform using Morris water maze test within the days which statistically at [F (5, 120) = 18.59, $p < 0.001$] between the groups and days [F (5, 120) = 18.59, $p < 0.047$]. The post-hoc test, however, shows that during the first day of the five days training, no significant difference was observed in the escape latency time between the groups ($p = 0.437$). Although the escape latency time decrease in the control when compared to the treated group on the second day, but still no statistically significant ($p = 0.086$) difference observed in location of the escape platform. From the third through the fifth day, the escape latency time showed some levels of consistency however not statistically significant ($p > 0.05$) difference between the groups, as they consistently had virtually equal escape latencies. On

the sixth day (probe), however, increase in mean escape latency time was observed in the treated

group when compared to the control which was statistically significant ($p = 0.001$).

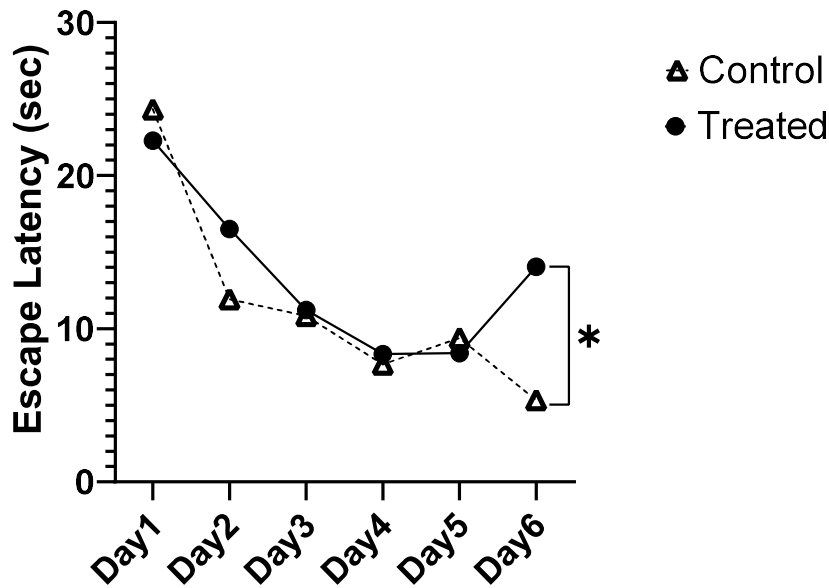


Figure 1: Escape latency time of Wistar rats at 12th week following prenatal exposure to 200 mg/kgbw hyoscyamine fraction of *D. stramonium* L. seeds during Morris water maze test. * $p = 0.001$

Figure 2 shows a percentage of mean exploration time in the sampling phase of novel object recognition test (NORT) using two identical objects (A1 and A2) between the controls and prenatally exposed, 200 mg/kgbw hyoscyamine fraction treated group. There was no statistically significant difference ($p > 0.05$) in the exploration time.

The result of test-phase (figure 3) in novel object an independent two samples – t -test for familiar and novel objects recognition tests (NORT) between controls and 200 mg/kgbw hyoscyamine fraction of *D. stramonium* L. seeds treated Wistar rats at gestation. The meantime took to explore the novel object (NO) were significantly ($p = 0.012$) higher than that taken for the familiar object (FO) in the

control group when compared to treated group. However, the meantime took to explore the novel object (NO) in the treated group was slightly higher compared to the familiar object (FO), there was, however, no statistically significant difference in the time spent to explore the two objects ($p = 0.325$).

Two samples t -test for discrimination index (figure 4) between the control and prenatally exposed, 200 mg/kgbw hyoscyamine fraction treated Wistar rats. The treated groups showed a significant decrease in discrimination ability when compared with the control ($p = 0.024$).

An independent two samples t -test for the novelty preference between control and prenatally exposed, 200 mg/kgbw hyoscyamine fraction of *D. stramonium* treated Wistar rats. Significant ($p = 0.022$) difference in the percentage novelty preference was observed between the groups as indicates in figure 5.

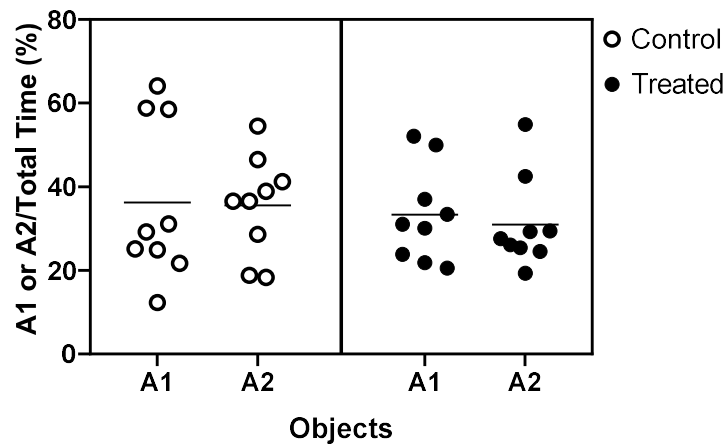


Figure 2. Sampling phase of Novel object recognition test of Wistar rats at 12th week after prenatal exposure to 200 mg/kgbw hyoscyamine fraction of *D. stramonium*.

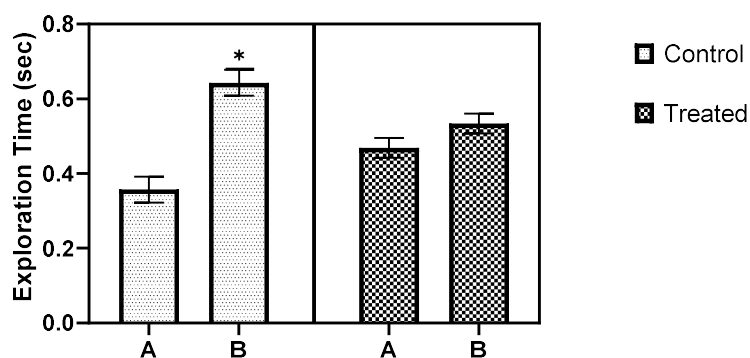


Figure 3 The test phase of Novel object recognition in Wistar rats at 12th week after prenatal exposure to 200 mg/kgbw hyoscyamine fraction of *D. stramonium* L. seeds during Morris water maze test. * $p = 0.001$

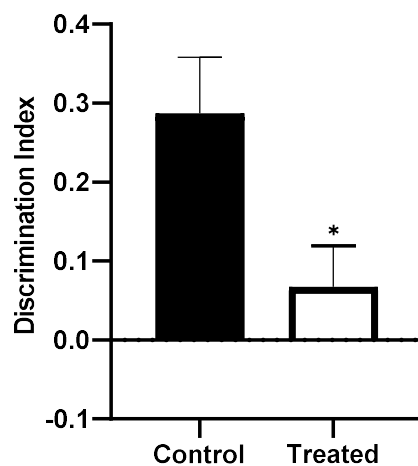


Figure 4. Discrimination index in Wistar rats at 12th week after prenatal exposure to 200 mg/kgbw hyoscyamine fraction of *D. stramonium* L. seeds during Morris water maze test. * $p = 0.024$

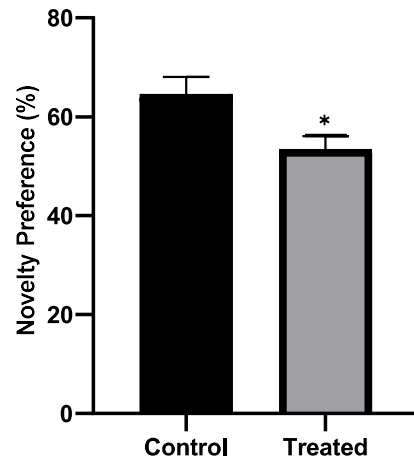


Figure 5. Novelty preference in Wistar rats at 12th week after prenatal exposure to 200 mg/kgbw hyoscyamine fraction of *D. stramonium* L. seeds during Morris water maze test.
* $p = 0.022$

Photomicrograph of CA1 region of adult Wistar rats at 12th week after being treated prenatally and normal saline from GD 15 – 20 respectively. The histology of the CA1 in the control group shows a normal architecture of the region featuring stratum oriens (SO) and stratum radiatum (SR). Comparatively, while in plate Vb of the treated

group showed hypochromic (white arrows) but a relatively thin region of the pyramidal cell layers (PCL). The region was poorly organized with scanty pyramidal cells (PC). Patches of cytoplasmic vacuolations (black asterisks) were also evident in the region.

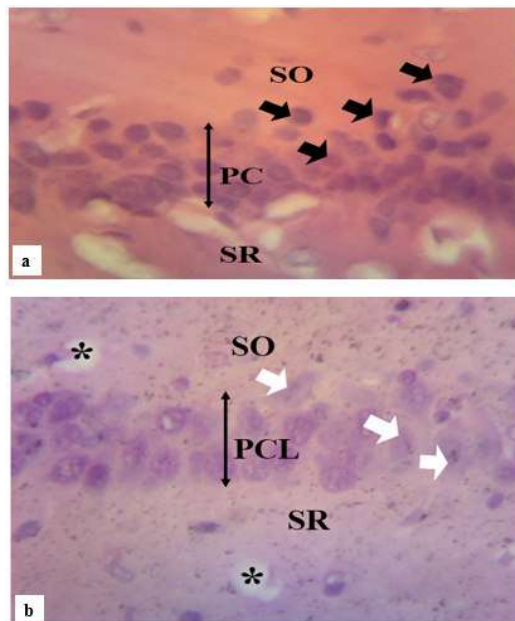


Plate V a&b: Photomicrograph of pyramidal cell (PC) region of the hippocampus in control Wistar rat at 12th week after treated with equivalent bodyweight normal saline (a) and 200 mg/kgbw hyoscyamine fraction of *D. stramonium* L. seeds (b)

from GD 15 – 20 (H&E, x 400). SO = Stratum oriens, SR = stratum radiatum, SLM = stratum lacunosum moleculare, = degenerated pyramidal cells, = cytoplasmic vacuolation (H&E, x 400).

Discussion

Tropane is believed to alter neurogenesis [40-41], but little knowledge is known as to the mechanisms of its actions. There was a significant increase in the escape latency time of the spatial learning memory in the treated when compared to the control group. This has probably indicated the destructive effect of the hyoscyamine fraction on the neural stem cells during the gestational stage of development. In a related study, loss of spatial learning and memory was seen in the Wistar rats treated with atropine at adolescence and reared till adulthood using a 'Y' maze [42]. These observations were similar to earlier studies of drug abuse effects on spatial memories in animals [43-45].

The results of the present study show that the novel object recognition (NORT) tests showed an increase in the mean exploration time, novelty preference and discrimination index time between a novel and familiar object in the control group compared with the treated group. A related study showed that atropine has an affinity for rapidly dividing cells [46-52]. This finding was similar to that of Olawepo *et al.* [42], which revealed a deficit in cognition following intraperitoneal injection of atropine particularly recognition memory in animals as compared with the control from the novel object recognition test (NORT) result. Also, it agreed with the findings of Joëls *et al.* [40] and Joosen *et al.* [41] which earlier reported that atropine impairs learning abilities and neurodegeneration. In a related study, atropine extract of Soma plant was shown to impair learning ability and neurogenesis [41]. *Datura metel* which contains tropane alkaloids induces hallucinations and metabolic disorder in rats [53-56], and have also been implicated in the disorders of hippocampal development in rats [27].

The CA1 contains pyramidal cells principally, and it plays an important role in matching and mismatching of information obtained from CA3 [57]. The present study shows that histology of the CA1 region of the treated group showed a comparatively hypochromic but a relatively thin region of the pyramidal cell layers with a poorly organized layer of scanty pyramidal cells. In a related study, delay in

the differentiation of the limbic system, hippocampus forming the peripheral border of the third ventricle at PND 0 of the hippocampus of litters treated with graded doses of ethanol extract of *D. metel* leaves also that the at PND 14, the pyramidal cell layer of treated groups appeared denser as compared to the control was reported [27]. The mild cytoplasmic vacuolation observed might probably result due to neural cell death [58] and gliosis in the area, which may be caused by the hyoscyamine fraction. *D. stramonium* tropane alkaloids all have a long duration of effect, cross the blood-brain barrier and have central anticholinergic effects [59] which induce strong hypnosis and can also induce neuronal degeneration [60].

Conclusion

Prenatal exposure to hyoscyamine fraction of *D. stramonium* by maternal oral ingestion impairs cytoarchitecture and cognitive function in the hippocampus of adult Wistar rats which may predispose neurodegenerative disease. Therefore, ingestion or exposure to tropane alkaloids related herbs should be avoided during pregnancy.

Acknowledgement

We sincerely appreciate Mallam Kabiru, Mallam Saidu Bala-riti, Bashir Sulaiman of the Departments of Pharmacognosy, Pharmacology and Pharmaceutical Chemistry for the assistance rendered in the course of this research. We equally appreciate the staff of the Animal House, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Science, Bayero University Kano, Nigeria for the animal breeding. We also thank Mr Jigo Dangude Yaro of histopathology unit of Ahmadu Bello University Teaching Hospital (ABUTH) for the slide preparation and interpretation.

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2Glutathione S-Transferase (Gstt1) Gene Polymorphism and Lipid Profile in Type 2 Diabetes Mellitus Patients Attending Murtala Muhammad Specialist Hospital Kano, Nigeria.

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Abstract

A cross sectional randomized, descriptive cross sectional study was conducted on the frequency of GSTT1 null alleles in patients diagnosed with type-2-diabetes mellitus (T2DM). A total of 40 patients with T2DM and 10 non-diabetic controls were included in the study. GSTT1 null-alleles genotyping was carried out using multiplex PCR amplification to amplify GSTT1 gene (460bp) while using β -globulin (250bp) as an internal control. The results showed that 55% of T2DM patients had BMI within reference limits, 13% are overweight. Additionally, patients with T2DM were found to have significantly higher ($p<0.05$) serum levels of glucose, total cholesterol, triglyceride and low density lipoprotein. Furthermore, the presence of null genotype of GSTT1 (deletion in GSTT1) was observed in 28% of diabetic patients. Subjects with GSTT1 deletion have significantly higher ($p<0.05$) levels of serum glucose, low-density lipoprotein and total cholesterol when compared with individuals without deletion (diabetic and non-diabetic). This results suggests that the deletion of GSTT1 gene might serve as a predisposing factor in the development of T2DM and dyslipidaemia

Keywords: Diabetes, Glutathione-S-Transferase, Lipid profile, PCR, Polymorphism.

Introduction

Diabetes Mellitus (DM) is a spectrum of metabolic disorders that is linked with impairment of metabolism and energy utilization from carbohydrates, lipids, and proteins. [1] Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes globally. About 451 million people are living with DM globally in 2017, and the figure is projected to increase by 35% in 2045. [2] DM is characterized by insulin resistance and relative insulin deficiency. [3] Obesity, hypertriglyceridaemia, low levels of high density lipoprotein cholesterol and hyperglycemia are major features of metabolic disease. The etiology of T2DM is not well understood, although in some individuals it is due to genetic and environmental factors. Multiple lifestyle and genetic factors contributes in the pathogenesis. [4] It is therefore important to study both genetic and environmental factors in understanding the aetiology

of this complex disorder. Moreover, it is believed that increased oxidative stress is one of the main factors in the etiology and complications of diabetes mellitus. [5] There is a relationship between oxidative stress and the progression of metabolic disorders diseases. [6] In addition, low antioxidant capacity increases the susceptibility toward diabetes complications. [7]

Glutathione transferase (GST) is one of the major cellular antioxidants enzymes that protects against environmental toxicants as well as reactive oxygen species (ROS) mediated cell injury. [8] ROS are generated through different biochemical pathways in diabetic conditions. One of the ways ROS are generated include the activation of the glycation reaction by the increase blood sugar in diabetes. Another way is by the activation of the membrane bound NADPH oxidase in the mitochondria. [8] ROS causes damage to lipids, proteins and nucleic acids,

by interacting with the macromolecules. ^[9] These interactions may lead to the development of age related chronic disorders. ^[10]

GST helps to detoxifies ROS, reduces peroxides and detoxifies most compounds through conjugation. ^[11] Polymorphisms of GST has been associated with different disorders, although its expression has been linked with oxidative stress, as observed in diabetic subjects. ^[11] Selective polymorphisms encoding **GSTM1** (Isoform of MU Class of GST) and **GSTT1** (Isoform of Theta Class of GST) genes may prove useful as genetic markers to recognize individuals with an increased risk in developing diabetic complications. ^[12] GSTT1-null genotype (Deletion in GSTT1 genes) predisposed patients to a higher risk for diabetic neuropathy in subjects with T2DM ^[13]. Studies by Geng *et al* ^[13] concluded that the GSTT1 null genotype was significantly more common in diabetic patients. There is paucity of information regarding the prevalence of GSTT1 among diabetic patient in Nigeria. This study is aimed at identifying GSTT1 genotype and lipid profile among diabetic and non-diabetic patients attending Murtala Muhammad Specialist Hospital Kano, Kano State., Northwest Nigeria

Materials and Methods

Study Participants

The study was conducted at Murtala Muhammad Specialist Hospital (MMSH), Kano. A total of 50 participants were recruited for the study;- 40 patients diagnosed with T2DM were included as cases. While individuals who were non-diabetic and who had no family history of diabetes were included as control. For both groups, only those who gave their consent were included. The ethical clearance for this research was obtained from Research and Ethics committee of the Ministry of Health, Kano State (MOH/Off/797/T. I/33).

Anthropometric Measurements: Height (m²) and weight (kg) were measured and Body Mass Index (BMI) were calculated using the Quetelet equation. BMI values below 18.5 were considered underweight. Individuals with BMI values within 18.5-24.9 were considered as normal, while those with values within 25.0-29.9 were overweight. Studied subjects with BMI between 30.0 -40.0 were considered morbid obese.

Inclusion Criteria for Patients: Patients who were diagnosed of type 2 diabetes mellitus were included as cases in the study and T2DM patients for the subject of study were confirmed by measuring the oral glucose tolerance test of selected individuals with plasma glucose level of ≥ 11.1 mmol/L.

Criteria for Control Recruitment: Individuals who were non-diabetic and who have never had any family history of diabetes were included as control. For both groups, only those who gave their consent were included.

Exclusion Criteria: Control subjects with any history of endocrine diseases, acute or chronic infection complication and those who did not give their consent in both groups were excluded. Also pregnant women were excluded.

Informed Consent: Informed consent for the study was obtained from each subject before enrolment in to the study. Strict confidentiality of patient record was maintained at all levels in the conduct of the study.

Blood Sample Collection and Processing

From each study participants, 5 mls of venous blood sample was collected using a sterile 21G needle fitted with syringe in fasting condition in the morning hours. Using standard technique of venipuncture, a vacutainer system was used to collect blood samples from the antecubital vein, 3 mls into EDTA container and 2mls into a fluoride oxalate container. These were used for assaying fasting blood glucose according to Trinder method. ^[14] Lipid profiles (total cholesterol, triglyceride, high density cholesterol and low density cholesterol) according to Wybeng *et al*. ^[15] The assays were carried out in the Biochemistry Laboratory of the Department of Biochemistry, Bayero University Kano.

DNA Extraction and Multiplex PCR Amplification

DNA was extracted using USB PrepEase® genomic DNA isolation kit (Cleveland, OH USA). The amplification was done using Promega PCR Master Mix (Promega M7502). Multiplex PCR was carried out in a 25µl reaction mixture, including 2.0µL of DNA, 0.51µl each of forward and reverse primers of both the GSTT1 gene and the β-globin gene. The β-globin gene was amplified to serve as positive

control to the PCR reaction. The PCR program was performed with an initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 60 s, and extension at 72°C for 30 s, and a final extension at 72°C for 10 min. The amplicons were then separated on 1.5% agarose gel electrophoresis and analysed for the presence or absence of the band in both diabetic and non-diabetic individuals. DNA ladder of 100bp (DNA Marker, New England Biolab. USA) was used to indicate the size of the separated amplicons. The method of Abdel-Rahman *et al* [16] was adopted. The multiplex PCR and gel electrophoresis were carried out in the molecular Biology Laboratory, Department of Biochemistry, Bayero University Kano, Nigeria.

Statistical Analysis:

Each parametric value was analyzed using descriptive statistics, to generate mean and standard deviation. The results of anthropometry and biochemical parameters obtained from diabetic patients were compared with those of controls using student t-test. One-way ANOVA was used in comparing the means obtained between the Subjects

and Control. *p*-values were two-tailed and a value of <0.05 was considered statistically significant. All analyses were performed using standard statistical software package of social science (SPSS) version 26.0 statistical analysis software.

Results

Results showed that among participants with T2DM, -55% have a normal BMI, 2.5% have morbid obesity, 32.5% are overweight while 10% are obese (Table 1). On the other hand, none of the control participants had morbid obesity, while 70% have normal BMI and 20% are overweight as shown in Table 1. The glucose, total cholesterol, triglyceride and low density lipoprotein levels were significantly higher ($p<0.05$) in diabetics compared to their non-diabetic counterparts (Table 2). The distribution of GSTT1 genotype among study participants in relation with the clinical parameters is presented in Table 3. Results showed that diabetic patients with deletion of GSTT1 (GSTT1 Null) had significantly ($P<0.05$) raised level of low density lipoprotein (LDL) compared to diabetic patients without the deletion of GSTT1. Total cholesterol and Glucose levels were slightly elevated in diabetic patients with GSTT1 deletion compared to diabetic patients with the presence of GSTT1.

Table 1: BMI category of study subjects

SUBJECT	Normal BMI	Overweight	Obese	Morbid obesity
Diabetic	22(55%)	13(32.5%)	4(10%)	1(2.5%)
Non-Diabetic (Control)	7(70%)	2(20%)	1(10%)	0(0%)

Table 2: Mean glucose and lipid profile levels of study subjects

Subject	TC(mmol/l)	TG(mmol/l)	HDL(mmol/l)	LDL(mmol/l)	GLU(mmol/l)
Diabetic	5.36±0.58 ^a	1.71±0.80 ^b	1.31±0.39 ^c	3.22±0.66 ^d	9.62±1.87 ^e
Non-diabetic (Control)	4.94±0.43 ^a	1.01 ±0.34 ^b	2.17±0.33 ^c	2.27±0.48 ^d	4.36±0.66 ^e

TC =total cholesterol; TG= triglycerides; HDL-C=high density lipoprotein cholesterol; LDL-C= low density lipoprotein. Values with similar superscript in the same column indicate significant difference ($p<0.05$).

Table 3: GSTT1 genotype among study subjects in relation with clinical parameters

GSTT1 Genotype	Glucose (mg/dl)	TC (mmol/l)	Triglycerides (mg/dl)	(LDL) (mmol/l)	BMI
Diabetic with deletion (n=11)	9.27 ± 1.18	5.70 ± 0.52 ^(a)	1.61 ± 0.63	3.59 ± 0.61	25.09 ± 4.45
Diabetic without deletion (n=29)	9.76 ± 2.07	5.24 ± 0.57 ^(a)	1.75 ± 0.86	3.15 ± 0.65	24.65 ± 5.49
Control with deletion (n=3)	4.27 ± 0.15	5.11 ± 0.17	1.06 ± 0.56	2.46 ± 0.17	23.38 ± 4.24
Control without deletion (n=7)	4.40 ± 0.80	4.88 ± 0.50	0.99 ± 0.28	2.25 ± 0.59	23.20 ± 5.14

Values with similar superscripts in the same column indicate significant association at $p < 0.05$

From Figure 1 the presence of GSTT1 genes was detected by presence of bands at 460bp while absence of these bands indicates a null genotype. Presence of GSTT1 gene product showed a band at 460bp for sample lane 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 15, 17, 18, 19, 20, 21, 23, 26, 27, 28, 29, 30, 31, 32 and

33. The deletion of GSTT1 was observed in lane 4, 9, 12, 13, 14, 16, 24, 25, and 34. The β -globulin amplified product (250bp) as an internal control is present in all the lanes. No band could be seen in lane 22, an indication that the PCR failed.

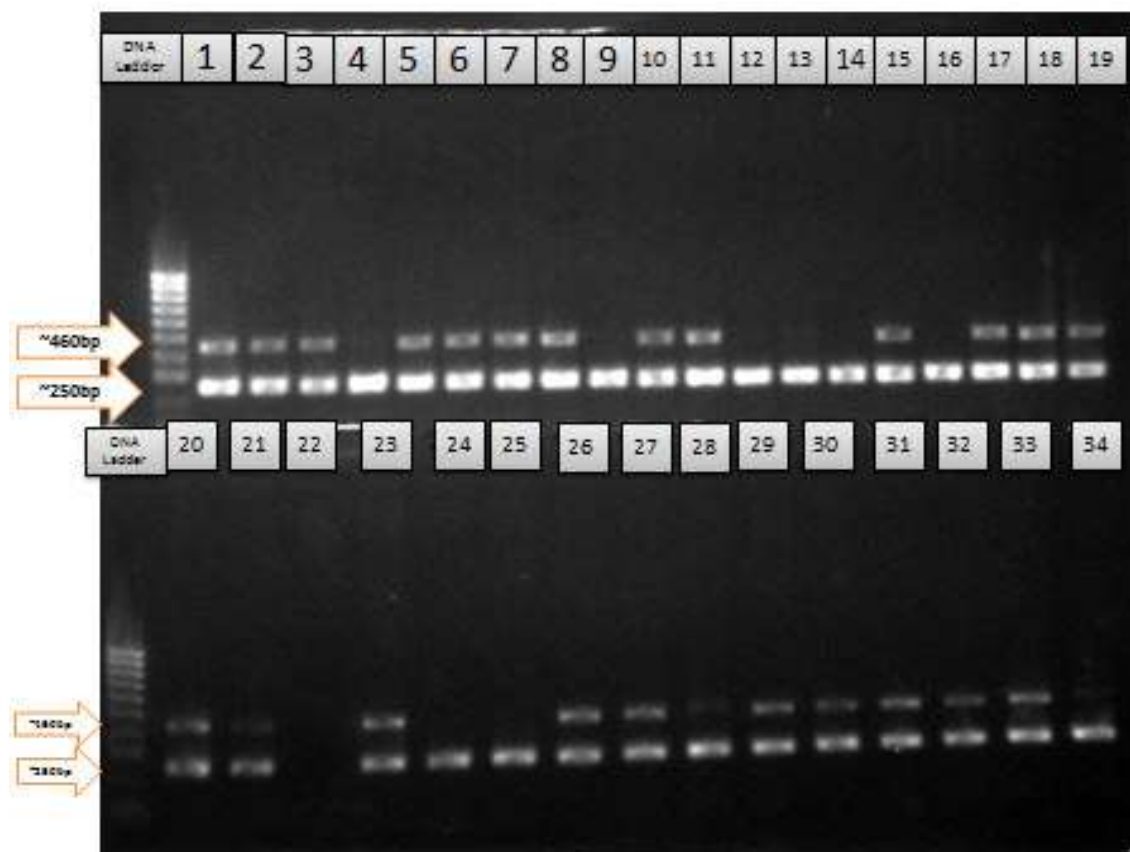


Figure1: Agarose gel electrophoregram of amplified PCR products. Lane 1-19 (Upper part of the gel electrophoregram) and Lane 20-34 (Lower part of

the gel electrophoregram). DNA ladder (100bp) is clearly indicated in both part of the gel electrophoregram.

Discussion

The observation that 55% of diabetic patients have normal BMI within reference limit while 13% are overweight results contradicts the well-known fact^[17] that diabetic patients usually are overweight or obese. The reason for this observation might be due to the proper management and dietary control the patients are receiving from the hospital. There were significantly higher levels of serum glucose, total cholesterol, triglyceride and low density lipoprotein among the diabetic patients when compared with the non-diabetic participants. Dyslipidemia is found in 85% of patients with diabetes.^[18] There is a clear evidence of dyslipidemia among the studied diabetic patients. Although, the underlying pathophysiology of lipid abnormalities has not been fully understood.^[19] The HDL levels in non-diabetic control were significantly higher than the diabetic patients in this study. This is in-line with several findings^{[20], [21], [22]} that the LDL cholesterol is associated with disease state, and may serve as a predisposing factor in the development of T2DM. A study among Chinese population shows a similar lipid profile pattern noted in diabetic patients.^[23]

In the present study, the presence of null genotype of GSTT1 (deletion in GSTT1) was observed in 28% of diabetic patients. This observation contradicts previous studies^{[24], [25]} that associate the deletion of GSTT1 with the development of diabetes. Furthermore, it was observed that diabetic patients with deletion of GSTT1 had increased levels of total cholesterol and LDL when compared with diabetic patients with the presence of the GSTT1 gene. This is not in-line with a study conducted by Al-Barqaawee *et al*,^[26] in which the fasting blood glucose and triglycerides were significantly higher in individuals with null GSTT1 than in those with GSTT1 genotype. It is not surprising that the raised levels of LDL is noticed among those with the deletion, even though strong association could not be established. The higher levels of total cholesterol and LDL serve as a risk factor for the development of dyslipidemia and hence a risk factor for the development of T2DM.^[27] In an Iranian study conducted by Pourkeramati *et al*,^[28] there was a significant association between GSTT1-null polymorphism and dyslipidemia. Among non-diabetics, GSTT1 deletion was observed in 30% of

the participants and those with deletion had increased levels of total cholesterol and low density lipoprotein (LDL) when compared with participants with the presence of the gene. This finding also points towards the assertion that the deletion of GSTT1 might be linked with the increased levels of LDL which could be associated with the risk of developing diabetes mellitus. Recently, study by Hashemi-Soteh *et al*,^[12] suggests that GSTT1-null genotype predisposed patients to a higher risk for T2DM complications.

The serum blood glucose was higher among the study participants (diabetic and non-diabetic) with deletion of GSTT1 when compared with their counterparts. Although the observed differences were not significant, the findings could serve as a guide in understanding associated risk among diabetic patient with GSTT1 null genotype. This study suggested that the GSTT1 might be a candidate genotype in predicting those with higher risk of developing DM. It can also be used in predicting the risk of developing increased lipid profile levels. Other polymorphic genetic variants possibly involved in DM could be added to a genetic panel for identifying patients at higher risk of developing complications and those that will benefit from more aggressive therapeutic lifestyle modification and pharmacotherapy.

Conclusion

The present study suggests that GSTT1 gene deletion may contribute to the development of T2DM in Nigerian population. The study also found that GSTT1 gene deletion is associated with a higher serum level of LDL. It also shows that presence of the gene may be associated with a low serum blood sugar. A positive link was also found between deletion of the GSTT1 gene and high serum level of total cholesterol in both diabetic and the control participants.

Ethics statement

The ethical clearance for the study was obtained from Research and Ethics Committee of the Ministry of Health Kano State and it was conducted according to the ethical principles for medical research involving human participants. (MOH/Off/797/T. I/33).

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Evaluation of the Effect of Clove Supplementation on Neurobehavioral Outcomes and Oxidative Stress Profiles in Wistar Rats Exposed to Low Level of Lead

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Abstract

Heavy metals, including lead, have been known to negatively impact various neurological functions. Experimental evidence shows that exposure to lead is associated with learning and memory impairment in animal models. Clove oil has been used as remedy in phytomedicine, and the oil has been shown to improve cognitive deficit in rats. This study was designed to test the hypothesis that clove supplementation in diet will modulate neurobehavioral impairment and oxidative stress induced by lead exposure in Wistar rats. Twenty (20) apparently healthy male Wistar rats were divided into four (4) groups (n=5). Group I served as normal control (water only) while group II served as negative control (lead only). Animals in groups III and IV were administered clove powder supplement in diet (3% and 6%) for twenty eight (28) days, along with lead. Neurobehavioral impairment was induced by oral administration of lead (10 mg/Kg) for 28 days. Pain perception was assessed using tail flick test and formalin induced pain. Tail suspension and open field test were used to assess depression, while memory was assessed using elevated plus maze. Exposure to lead decreased central pain perception, increased depressive symptoms and impaired spatial memory, as well as increase oxidative stress, but these effects were reversed by dietary clove supplement. This study demonstrates that dietary clove supplement improve neurobehavioral deficits and oxidative stress status in Wistar rats exposed to low level of lead.

Keywords: Clove; Lead; Pain; Depression; Memory; Supplement

Introduction

Neurobehavioural disorders produced by lead exposure has been a focus of research for a while. Exposure to lead has been shown to be associated with learning and memory disorders.^[1] Lead, a highly poisonous metal, affects almost every organ in the body, with the nervous system being the most severely affected.^[2] It exists in different forms and is a constituent of a variety of organic compounds, with ability to directly penetrate the skin and respiratory tract. Exposure to lead occurs mainly by ingestion and inhalation from petroleum products, leaded paints, contaminated air, water, soil, food, from where it enters the blood and affect the central nervous system.^{[3][4][5]} Blood lead level as low as 5 µg/dl has been reported to be toxic. Lead toxicity has been reported to cause reduction in N-

acetylaspartate, leading to increased neuronal loss in the frontal lobe and hippocampal glia cell dysfunction, conditions that are associated with impaired intelligence quotient (IQ), academic skills, executive functions, fine and gross motor skills, memory and language.^[6] Early signs of lead toxicity in children include subtle neurobehavioural deficits which adversely affect classroom performance and social interaction, as well as vague gastrointestinal and CNS complaints in adults.^[7] Lead neurotoxicity occur mainly as a result of lead-induced oxidative stress (directly or indirectly), alteration in membrane bio-physics, dysregulation of cell signalling and impairment of neurotransmission. The endothelial lining of the blood-brain barrier are exposed to the toxic effect of lead as it crosses into the brain, with astrocytes being highly sensitive at low

concentration.^[8] Lead is able to cross the blood-brain barrier due to its ability to substitute for calcium ion.

Syzigium aromaticum is a tree that belongs to the family Myrtaceae. It is native to Indonesia but it is also commercially harvested in India, Pakistan, Sri Lanka, Comoro Islands, Madagascar, Seychelles, and Tanzania.^[9] Its parts, including flower buds, leaves, and stems are used as remedies in phytomedicine. Clove, the flower bud of *Syzigium aromaticum*, is commonly applied to gums for treatment of toothache, and as analgesic during dental surgery. It possesses antibacterial properties and is used to cleanse bacteria in various dental solutions, and also to relieve gum pain.^[10] Clove is also used as flavouring agent in foods and beverages, as well as in manufacturing industries to make toothpaste, soaps, cosmetics, perfumes, and cigarettes. Clove contains vitamins B and C and is 10% volatile oils, mostly eugenol, a substance that has anaesthetic effects, and improves feed and water consumption of broiler chicks exposed to heat stress,^[11] though a higher concentration was found to have a negative effect on the broiler chick performance.^[12] According to Mahrous et al.,^[13] clove supplementation enhances serum immunoglobulins, INF- γ , and IL-10, improved antioxidant potential as well as increased immunity and antioxidant activity in broiler chickens. Clove has been reported to contain many biologically active compounds, such as eugenol, β -caryophyllene, phlobatannins, reducing sugar, steroids, tannins, phenols, glycosides, and made to minimise animal suffering.

flavonoids.^[14] In rats, clove has been shown to enhance functions of the liver and kidney, improve antioxidant status and protect against H₂O₂ induced cell damages,^[15] as well as improve cognitive performance.^[16] In this study, we investigated the effect of clove powder supplementation on neurobehavioral performance of Wistar rats exposed to low dose administration of lead.

Materials and Method

Experimental animals

A total of twenty (20) apparently healthy male Wistar rats were obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria, Kaduna state. The animals were housed in plastic cages under normal laboratory conditions and were acclimatized for one week before the commencement of the experiment. The experimental animals weighed 100-150g. Animals were treated according to the guidelines established by the Ahmadu Bello University committee on animal care and use, with free access to feed and tap water. The animals were divided into four (4) groups (n = 5), as shown in Fig. 1. Lead was dissolved in distilled water and administered orally at a dose of 10 mg/Kg/day. Dried clove was ground into powder and mixed as a percentage of feed. Treatment with lead and clove were carried out concomitantly and lasted for twenty eight (28) days before commencement of the experiments. Handling and testing were performed during the day (10:00 am to 12:00 noon). All efforts were

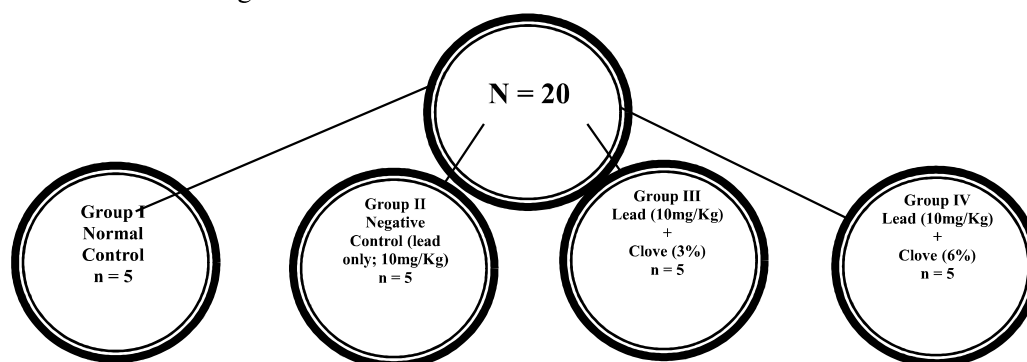


Figure 1: Animal grouping

Behavioural Study

All experiments commenced at 29th day of the study.

Tail flick test

The tail flick/tail immersion test was conducted following the method described by Oyebanji et al.^[17]. The terminal 2 cm of the rat tail was first immersed in water at room temperature, and then in a 500 ml beaker containing water maintained at $55 \pm 1^\circ\text{C}$ using water bath. A thermometer was placed inside the water to monitor the temperature. The responses to thermal pain was determined as time taken for the animal to flick its tail (in seconds).

Formalin induced pain

Formalin induced pain was evaluated by injecting 50 μl of freshly prepared 2.5% formalin solution into the sub-plantar surface of each rat.^{[18][19]} After formalin injection, The rats were placed individually in an observation chamber and observed after 5 min, and then, at the end of 45 min. The severity of pain response was recorded for each rat based on the following scale: (0) rat walks or stands firmly on the injected paw; (1) the injected paw is favoured or partially elevated; (2) the injected paw is clearly lifted off the floor; (3) the rat licks, chews, or shakes the injected paw.

Tail suspension test

The tail suspension test is a widely used model to assess despair like behaviour in rats. It is based on the principle that animals subjected to short term, inescapable stress of being suspended by their tail will develop an immobile posture that represents depressive behaviour. The amount of time the rats spend immobile is recorded. Cut off time is 6 minutes. In the present study, the apparatus consisted of a wooden box ($54 \times 30 \times 52\text{ cm}$) with a hook in the centre of the ceiling.^[20] Each rat was individually suspended by the tail from the hook with an adhesive tape and immobility time was recorded (in seconds).

Open field test

The open field test is being used to measure locomotor activity as an index of depression. The apparatus for open field test is a square ($76 \times 76\text{ cm}$) with opaque walls 42 cm high. The floor is divided into 16 equal squares. To monitor the activity animals taken out from their home cages were

placed in a square at a corner of the open field (one at a time). Numbers of squares crossed with all four paws were counted as number of line crossing and number of center crossing with all four paws were also recorded for 5 minutes. The number of center crossing and line crossing were used to assess depression.^[20]

Elevated Plus Maze (EPM)

The elevated plus maze apparatus used in the present study consisted of two open arms ($50 \times 10\text{ cm}$) crossed with two closed arms of the same dimensions with walls 40 cm high. The arms were connected with a central square ($5 \times 5\text{ cm}$) to give the apparatus a plus sign appearance. The maze was elevated 60 cm above the floor. On the acquisition trial, the rats were placed individually at the end of the open arm, facing outward. The time taken by the animal to move from the open arm to either one of the enclosed arms was recorded (in seconds). Cut off time was 90 seconds. To become acquainted with the EPM, the rats were allowed to explore the apparatus for 20 seconds after entering the enclosed arm before being returned to their home cage. The same procedure was repeated after 24 hours for the retention trial. A longer transfer latency (TL) period to reach the enclosed arm on the second trial indicated poor memory. The apparatus was wiped clean with 70% ethanol before testing each animal.^{[21][22]}

Biochemical analysis

At the end of the behavioural test, the animals were anaesthetized by intraperitoneal injection of Ketamine (100 mg/Kg) and Diazepam (5 mg/Kg). Four (4) ml of blood was collected in plain sample bottles via cardiac puncture. The blood was allowed to clot and the serum was collected and used for analysis of Superoxidase dismutase (SOD), Glutathione peroxidase (GPx) and Malondialdehyde (MDA) concentration using colorimetric method. SOD activity was determined by the method described by Martin *et al.*, (1987). Auto oxidation of heamatoxylin (with increase of absorbance at 560nm) is inhibited by SOD activity at pH 7.8; the percentage of the inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of heamatin at 560nm. To

920µL of phosphate buffer (0.05M, pH 7.8) was added 40µL of sample. A reagent test was also prepared by replacing the sample with 40µL of sample dilution buffer (0:85% NaCl). The mixture was incubated for 2 minutes at 25°C before the addition of 40µL of heamatoxylin. Absorbance of the sample test and reagent test was read at 560nm immediately and after 5 minutes against the sample blank (distilled water).

Glutathione peroxidase (GSH-Px) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide. 100 µL of enzyme sample was incubated for five minutes with 1.55 ml stock solution (prepared in 50 mM Tris buffer, pH 7.6 with 0.1 mM EDTA) containing 0.25 mM GSH, 0.12 mM NADPH and 1 unit glutathione reductase. The reaction was initiated by adding 50 µL of cumene hydroperoxide (1 mg ml⁻¹), and the rate of disappearance of NADPH with time was determined by monitoring absorbance at 340 nm. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 µmol of NADPH to NADP per minute. Results are expressed as units mg⁻¹ protein.^[24]

Thiobarbituric acid reactive substance (TBARS) in the tissue was estimated using the method described by Rukmini *et al.*, (2004). The formation of malondialdehyde is the basis for the well known TBA method used for evaluating the extent of lipid peroxidation. At low pH of 2-3 and high temperature (100°C), malondialdehyde (MDA) binds thiobarbituric acid to form a pink complex which absorbs maximally at 532nm. To 0.5ml of sample, 0.5ml saline and 1.0ml of 10% TCA was added. 0.25ml of 0.1M TBA was also added to the mixture. The mixture was incubated for 1 hour at 95°C, cooled and centrifuged at 3000rpm for 20 minutes. The absorbance of the pink colour produced was read at 535nm.

Statistical analysis

Results were presented as mean ± standard error of mean (SEM). All analysis were carried out using SPSS version 23 (SPSS Inc. Chicago. US). Differences between groups were analyzed by one

way analysis of variance (one way ANOVA), followed by Tuckey's *post hoc* test.

Results

Figure 2 shows the result of effect of clove supplement on tail flick latency of the tail immersion test in lead induced neurotoxic Wistar rats. Animals in the negative control group had the highest mean tail flick latency (2.35±0.20), while those that were treated with 3% clove supplement had the least mean tail flick latency (1.85±0.06). Animals in the control group and those treated with 6% clove supplement had mean tail flick latency of 2.13±0.19 and 2.04±0.23 respectively. Mean differences between all the groups were not statistically significant ($P = 0.31$). Effect of clove supplement on mean pain score of formalin-induced pain in lead induced neurotoxic Wistar rats showed that pain score at 5 minutes was not statistically significant ($P > 0.05$), although animals in the negative control (untreated) group had the highest pain score (lowest pain perception), while those treated with 6% clove supplement had the least pain score (highest pain perception) (figure 3). Result of formalin-induced pain perception at 45 minutes, on the other hand, showed that animals treated with 6% clove supplement had the highest mean pain score (least pain perception) compared to all the other groups, and the differences were statistically significant ($P = 0.004$) (figure 3). Result of the effect of clove supplement on tail suspension test showed that there was no statistically significant differences in the mobility and immobility time between the groups ($P > 0.05$). Animals in the negative control group were found to have the highest mean immobility time (196.60±19.89) and the lowest mean mobility time (163.4±19.89). Animals that were administered with 6% clove supplement were found to have a significantly higher mean mobility time (230.00±16.70) than immobility time (130.00±16.70) ($P = 0.003$) (figure 4). Result of open field test showed that mean number of line crossing was higher in the 3% group (68.20±13.23) when compared to all the other groups, and the difference was statistically significant ($P < 0.05$) (figure 5). Result of elevated plus maze outcomes showed that the mean latency to enter the closed arm in the first phase (acquisition) of the test was significantly higher in the lead only treated group ($P < 0.05$)

whenc compared to all the other groups. Animals in the negative (lead only) control group have the highest mean latency to enter the closed arm (88.00 ± 2.00) during the second phase (retention) of the test, and the differences were statistically significant ($P < 0.05$) when compared to all the other groups. Animals in the negative control group were found to have a higher retention time than acquisition time, while animals in all the other

groups had lower retention than acquisition time, though the difference is only statistically significant in the normal control group ($P = 0.03$) (figure 6). Lead administration causes increase in SOD and GPx activities, but their level was reduced by concomitant treatment with clove supplement (figure 7). Clove supplement at 6% of diet also decreased the level of serum MDA (figure 8).

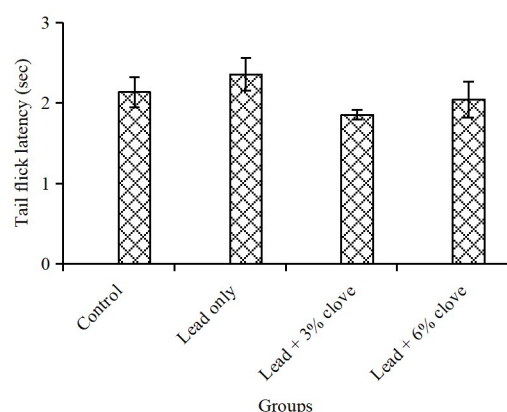


Figure 2: Effect of clove supplement on tail flick latency of tail immersion test in Wistar rats exposed to low dose of lead.

^aMean differences was significant compared with negative control ($p < 0.05$; One way ANOVA)

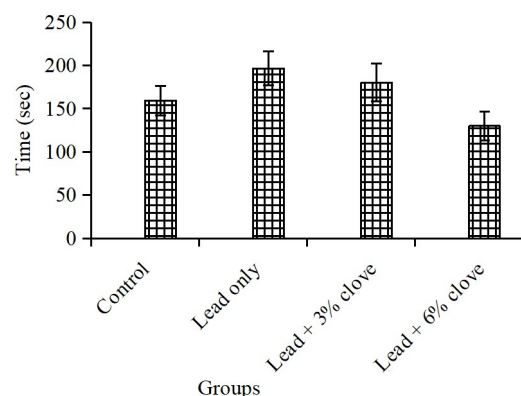


Figure 4: Effect of clove supplement on immobility times of tail suspension test in Wistar rats administered low dose of lead.

^{*}Statistically significant difference compared to negative control group ($P = 0.003$; ANOVA)

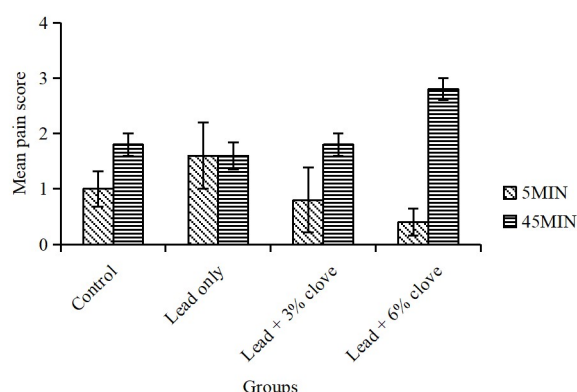


Figure 3: Effect of clove supplement on mean pain score at 5 and 45 minutes of formalin induced pain in Wistar rats exposed to low dose of lead.

^{*,#}Statistically significant at 5 minutes between groups ($P < 0.05$; One way ANOVA)

^{a,b}Statistically significant at 45 minutes between groups ($P < 0.05$; One way ANOVA)

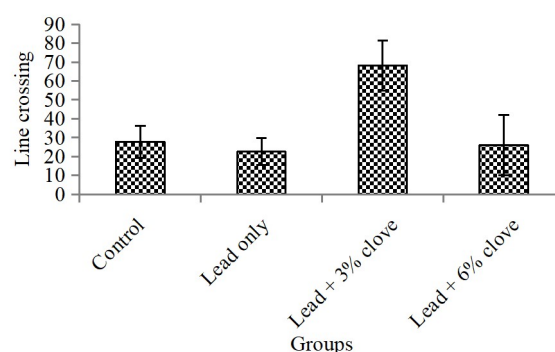


Figure 5: Effect of clove supplement on mean number of line crossing of open field test in Wistar rats administered low dose of lead.

^{a,b}Bars with different superscript letters are statistically significant ($P < 0.05$; ANOVA)

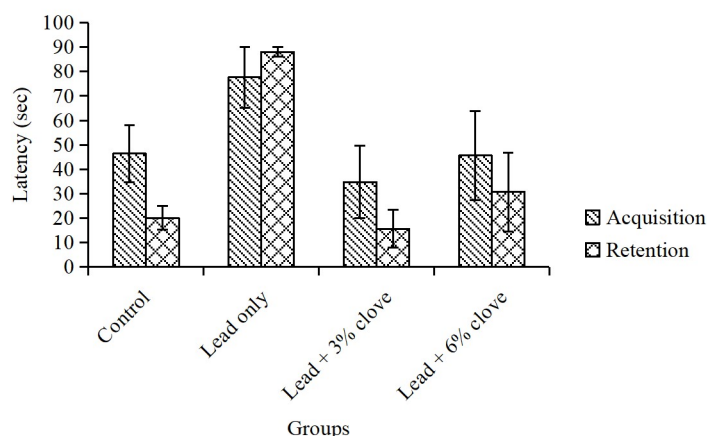


Figure 6: Effect of clove supplement on mean latency to enter closed arm of elevated plus maze (EPM) in Wistar rats administered low dose of lead.

^{a,b}Statistically significant difference between groups ($P < 0.05$; ANOVA)

^{*,#}Statistically significant difference between acquisition and retention within the same group ($P = 0.03$)

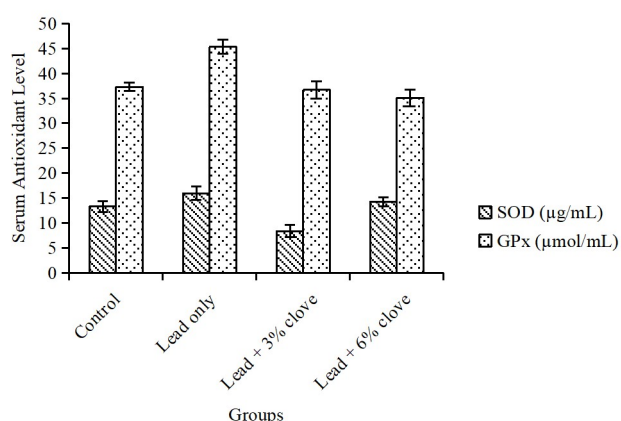


Figure 7: Effect of clove supplement on antioxidant enzymes in Wistar rats administered with low dose of lead.

^{*,#}Statistically significant difference compared to control (ANOVA; $P < 0.05$; One way ANOVA)

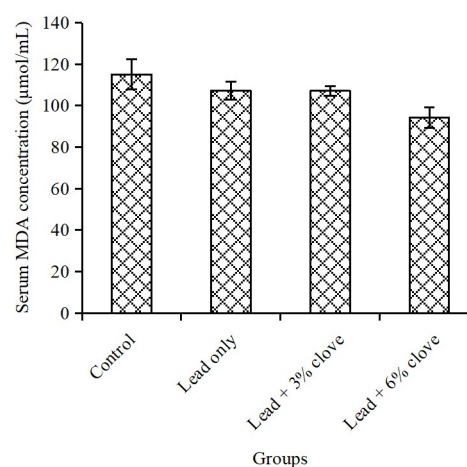


Figure 8: Effect of clove supplement on serum MDA concentration in Wistar rats administered low dose of lead.

^{a,b}Mean difference is statistically significant ($P < 0.04$; One way ANOVA)

Discussion

Exposure to lead can cause pathophysiological changes in several organ systems, including central nervous system, renal system, hematopoietic system, and immune system.^[7] This study was carried out based on the hypothesis that long term exposure to low concentration of lead has no significant toxic effect on the central nervous system. Animals were exposed to lead at a dose of 10 mg/Kg and treated with clove supplement as 3% and 6% of diet concomitantly for twenty eight (28) days. Minimal toxic level of lead has been reduced over time and its toxicity is organ specific.^[8] Exposure of the Wistar rats to 10 mg/Kg lead for 28 days in the present study caused an increase in tail flick latency in the

tail immersion test, indicating a decrease in central pain perception, but the effect was reversed due to concomitant administration of dietary clove supplement. This suggests sensory perception deficit due to long term exposure to lead, and its modulation by dietary clove supplement. Mean pain score at 5 minutes of formalin induced pain after chronic lead administration in Wistar rats was found to be decreased by clove supplementation, indicating yet another effect of clove on central nociceptive perception. Mean pain score at 45 minutes was increased by 6% clove supplementation, showing that at high concentration, clove may have peripheral analgesic effect after chronic lead exposure. The antinociceptive and anti-inflammatory

effects of clove have been reported in previous studies.^{[25][26][27][28]} Analgesic effect of clove is postulated to be due to the action of eugenol on vanilloid receptors, as the hydrocarbon tail in eugenol, in combination with the polar OH group on the ring allows it to interact with vanilloid receptors,^{[29][30]} especially TRPV1 and TRPV3 channels.^[31] The present study also supports the traditional claim on the use of clove or clove oil to relieve pain.^{[32][33]} The observed peripheral analgesic effect of clove may be due to the anti-inflammatory property of eugenol, an essential ingredient in clove. Essential oil of clove has been reported to possess anti-inflammatory activity on carrageenan induced paw oedema in rats.^[34] The potent anti-inflammatory property of eugenol has been associated with its ability to inhibit activity of COX-2 enzyme, likely through inhibition of PGE2, suppression of the nuclear factor kappa B (NF-κB) pathway and inhibition of interleukin-6 (IL-6) production.^[35] Eugenol has also been reported to produce a reversible, dose-dependent anesthetic effect in male Sprague-Dawley rats.^[36] Antinociceptive effect of eugenol has also been proposed to be via its action on P2X receptors in trigeminal ganglion neurons.^{[31][37][38]}

Lead administration, in the present study, was found to increase depressive symptoms in the Wistar rats, as evident by increase in immobility time during the tail suspension test, as well as a decrease in mean number of line crossing during the open field test. Clove supplementation, on the other hand ameliorated the depressive symptoms, as evident in the lower mobility time in the 6% and higher line crossing in the 3% supplement groups. A previous study reported an association between increasing blood lead level and risk of major depressive disorder in young adults.^[39] Another study by Jaffar et al.^[40] reported significant reduction in immobility response by clove aqueous extract. Clove essential oil was reported to significantly decrease immobility time of forced swimming test in MnCl₂ exposed rats,^[41] suggesting an antidepressant-like effect. The mechanism of lead induced depression may be related to its ability to disrupt the catecholaminergic and serotonergic systems. Experimental evidence showed that lead exposure result in decrease in the activity of tyrosine hydroxylase in the brain, resulting in decrease in brain catecholamine

concentration.^[42] It also alters tryptophan metabolism, and causes excessive accumulation of 5-hydroxyindole acetic acid, a serotonin metabolite, in the brain.^[43]

Clove supplementation, in the present study, significantly improved spatial memory impaired by chronic administration of low dose of lead. This is evident by the decrease in latency to locate the closed arm of EPM model of spatial memory in Wistar rats. Administration of lead significantly increased the latency to locate the closed arm both in the acquisition and retention phase, an effect that was prevented by concomitant administration of clove supplement in diet. As a simple method that evaluates spatial memory,^[44] the EPM was used initially to test for anxiety,^[21] but is also widely employed to evaluate effects of drugs on memory.^{[45][46]} The nervous system, as compared to other body systems, appears to be the most sensitive to lead induced toxicity, leading to dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucinations.^[47] Exposure to lead has been reported to result in significant neurophysiological deficit, including prominent defect in visio-spatial memory^[48] and decreased intelligence,^[49] as observed in the present study. Lead induced neurotoxicity involves a wide range of mechanisms that include apoptosis, excitotoxicity, interference with neurotransmitter metabolism, alterations in second messenger systems and damage to mitochondria.^[50] Toxic effects of lead has been attributed to its ability to replace calcium, and disruption of dopaminergic and glutamatergic systems, mechanisms that can be associated with disordered motor control and impaired cognition. Introduction of clove supplement in the diet of the animals at a concentration of 3% and 6% significantly improved cognitive behavior impaired by lead. The finding of this study corroborated previous finding by Halder et al.^[51] where treatment with clove oil significantly reversed scopolamine-induced cognitive deficit in mice. In another study, acute administrations of clove extracts was reported to enhance the learning and memory recall ability in mice in an inverse dose-dependent manner.^[52] Clove oil, which affect both cholinergic and glutamatergic systems, directly inhibits NMDA induced cell neurotoxicity.^[16]

Oxidative stress and lipid peroxidation has been known to play a significant role in cell damage. Serum MDA level was not significantly affected by chronic administration of low level of lead in the present study, an effect that was corroborated by the high levels of serum SOD and GPx. Treatment with clove supplement decreased serum MDA level, indicating a membrane stabilizing effect. On the other hand, serum levels of SOD and GPx were not increased by clove supplementation. Oxidative stress, a normal and necessary process that occur in the body, is an imbalance or disturbance between free radicals generation/accumulation and body's anti-oxidant defence system.^{[53][54]} Biomarkers such as MDA and GPx levels as well as SOD and CAT activities are commonly used to determine association between blood lead level and oxidative stress.^[55]

Conclusion

Exposure to low level of lead has been demonstrated to induce neurobehavioral deficits, as observed due to increase in pain threshold (decreased pain perception), increased depression symptoms as well as impairment in spatial memory. Clove supplement, on the other hand, has improve the observed neurobehavioral deficits induced by chronic low dose lead exposure.

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Conflict of interest

The authors declare no conflict of interest

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Relationship of Fingerprint Pattern and Body Mass Index (BMI) among Students of Basic Medical Sciences, Bayero University Kano

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Abstract

Fingerprint patterns are formed during a fetal period and are unique in each individual. The current study aimed to assess the predictability of body mass index (BMI) from the measurement of the fingerprint pattern. A cross-sectional study with a consecutive sampling of four hundred (400) consenting undergraduate students was the backbone of the current study. All the ten fingerprints of each of the 400 participants were captured and categorized as either Loops, Whorls, or Arches for each finger. The body mass index (BMI) was measured using standard anthropological techniques involving weight measurement in kilograms and height measurements in meters. The mean age of the participants was 21.86 ± 3.37 years. Loop fingerprints pattern was the most common in the participants (58.4%), followed by whorls (27.9%), and then the least was arches (13.7%). The frequency of the different categories of BMI revealed that the normal weight BMI category (67.4%) made up the largest proportion of study participants. In the distribution of the fingerprint patterns on the middle left and middle right fingers, the participants with normal weight BMI had the highest proportions of the loop, then whorl and then least proportion of arch, and these distributions were statistically significant. Whorl fingerprint pattern was significantly more abundant among obese participants. Further evaluation of the distribution of the fingerprint patterns in relation to the BMI of the study participants suggested that the association was mainly due to the difference the fingerprint patterns among the females and not the males.

Key words: Fingerprint, Body mass index, Consecutive sampling, Cross-sectional study, Prediction

Introduction

A fingerprint is defined by a set of ridgelines with their associated fingerprint valleys on the tip of the finger [1]. The ridgelines could run parallel, intersect, or sometimes terminate, thus forming two types of features known as ridge ending and ridge bifurcation. In a fingerprint image, ridges are dark whereas valleys are bright [2]. The points where the ridgelines terminate is also called minutiae[1,3,4]. The type, direction, and location of minutiae were the most commonly used features in the early studies of fingerprint patterns[5]. A good quality fingerprint contains 25-80 minutiae and they are one of the most unchanging, earliest, and easiest forms of signatures used in biometric[6,7]. Face and voice patterns are other phenotypic signatures that have been used, but they are altered with age[4]. Thus, fingerprints have found application in sensitive aspects of security and civil issues such as banking, as well as other activities that require granting passage to authorized

persons only[8]. Body mass index (BMI), is the ratio between weight and height (kg/m^2) and thus it relates a person's weight to his height as a normalized index[9]. It is commonly used in practice as a measure of body size, an estimator of obesity, and a popular heuristic approximation for body fatness in epidemiology and clinical practice across the globe [10]. The World Health Organization defined BMI-based categories as underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$), normal weight ($18.5\text{--}24.9 \text{ kg/m}^2$), overweight ($25.0\text{--}29.9 \text{ kg/m}^2$) and obese ($\geq 30.0 \text{ kg/m}^2$)[11].

The prevalence of obesity is increasing rapidly even at old ages as well as younger ages [12,13]. Its prevalence had grown rapidly in America, Europe, UK, other developed countries, and the world at large[13-15]. In a Nigerian study, a high prevalence of obesity (17%) and overweight (31%) was

reported among urban dwelling adults [16]. The prevalence among rural dwelling adult Nigerian was overweight (23.0%) while the pre-obese and obese groups made up 23% of the study population[17]. It is also a major risk factor for mortality, causing 3 million death each year worldwide and morbidity from cardiovascular disease, hypertension, type 2 diabetes, gallbladder disease, several types of cancers, and degenerative joint disease[18,19]. Obesity has also been associated with problems of fertility, pregnancy, and childbirth [20]. Overweight or obesity (i.e. over 25kg/m²) has a greater impact on the risk of disease for women than for men[21]. The risk of dying from diabetes among women that were obese was higher than for men that were obese [22]. Also, it is the third most important predictor of cardiovascular disease after age and blood pressure among women [23]. An earlier report revealed that the mortality ratio for all cancers was 1.33 times higher for obese men and 1.55 for obese women [22]. Individuals with a BMI that is lower than that considered to be desirable are also at increased risk of poor health [24].

The pathophysiology of obesity involves both genetic and environmental factors[25]. Numerous genetic variants that play some role in the development of obesity had been identified, yet it has become obvious that they do not explain the entire heritability and development of obesity. An epigenetic feature may be the necessary tools that could describe the heritability and development of obesity because their impact commences inside the womb during fetal development [26]. It is also postulated that epigenetic factors of obesity can influence fingerprint patterns since they are both associated with the fetal developmental period[27]. Thus, it may be used as markers of obesity throughout life [28]. The gap in the literature stems from the fact that there are no alternative phenotypic features that have been evaluated for a relationship

$$n = \frac{z^2 pq}{d^2}$$

Therefore, the minimum number of subjects needed for this study was 384.

Ethics Consideration and Informed Consent

Before the commencement of the research, the study protocol was assessed by the postgraduate committee of the department of Anatomy in Bayero

with the body mass index (BMI) which may serve as a predictor for BMI. Identifying a predictive relationship between BMI and fingerprint pattern can find good usage in the screening of neonates and young children that are at higher endogenous risk of developing obesity to allow appropriate dietary and physical adjustments to arrest the menace of obesity.

Materials and Methods

Materials

Computer (Laptop, Toshiba, China), Fingerprint scanner Device (Digital parsona, China), Data collection sheet (Proforma), USB (Toshiba, China), Digital weighing scale (Made in China), Measuring Tape and Pen.

Study Protocol

Study Location and Participants

The research was conducted in the Faculty of basic medical sciences, Bayero University Kano. The recruitment of study participants was carried out within four weeks (one month).

Inclusion Criteria

Participants from the Faculty of Basic Medical Sciences must be free from any deformity or pathology that affects the fingers.

Exclusion Criteria

Any participant from other Faculty other than the Faculty of Basic Medical Sciences and those that have deformity or pathology that affects the fingers.

Study Design / Sampling Method

A cross-sectional study with a consecutive sampling of consenting participants

Sample Size Determination

The sample size was determined using a standard formula Lwanga and Lemeshow, 1991:

Where;

$$n = \frac{(\pm 1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 384$$

University Kano for ethical compliance. Also, verbal informed consent of the entire participant was obtained.

Methodology

Paper towel cleaning of fingers was sufficient for removing dirt and grease that may interfere with clear and proper identification of fingerprint images. Imaging of the entire fingerprints pattern was done for both hands by firmly applying a finger on the scanner one at a time to allow the individual images to be properly labeled and saved. An application system designed by ZKTeco Inc. China was modified by a software programmer using Microsoft Visual Basic 6.0 integrated development enrollment (IDE) was used to capture the fingerprints using the scanner device (Digita Persona., China) when connected to the computer system via Universal Serial Bus (USB)^[29].

Height was measured as a vertical distance between the standing surface and the vertex of the head using a stadiometer. Weight of the subject was evenly distributed on the feet, the heels of the feet were placed together with heels touching the base of the vertical board, the buttocks, scapular, and head were positioned in contact with the vertical backboard^[30].

Weight of the subjects was measured using a digital weighing scale to the nearest 0.1kg when the subject was standing on the center of the weight scale

platform and removing all material that may interfere with the result of the research [30]. Body mass index: calculated as weight in kg divided by height in m² [31].

Statistical Analyses

Fingerprint, weight, height, body mass index (BMI), gender and age were noted for the whole study population. The qualitative data obtained were presented as proportion/frequency. Mean and the standard deviation was calculated for all the quantitative parameters. And a chi-square test was used to analyze the association between fingerprint pattern and body mass index (BMI), $P < 0.05$ was considered statistically significant. Data collected was analyzed using statistical package for sciences Solution (SPSS) software version 25, USA.

Results

A total of 399 study participants were recruited with 218 males and 181 females. The mean age, height, weight, and BMI of the study participants were 21.86 ± 3.37 years, 1.670.09, 58.1710.74, and 20.723.62 (Table 1). The mean height, age and weight were higher in males when compared to females and the differences was statistically significantly (Table 1).

Table 1: Descriptive statistics of quantitative variables among students of basic medical sciences Bayero University Kano.

	Variables	Minimum	Maximum	Mean SD
All study participants	Height (cm)	145.00	190.00	167.00 9.00
	Age (years)	18.00	40.00	21.86 $\pm$ 3.37
	Weight(kg)	37.7	123.40	58.1710.74
	BMI(kg/m ²)	12.60	41.71	20.723.62
Females	Height (cm)	146.00	190.00	163 8.00*
	Age (years)	18.00	40.00	21.09 3.71*
	Weight(kg)	37.70	123.40	55.68 2.64*
	BMI(kg/m ²)	12.60	41.71	21.05 4.56
Males	Height (cm)	145.00	188.00	172 7.00*
	Age (years)	18.00	35.00	22.54 2.77*
	Weight(kg)	39.80	97.70	60.25 8.35*
	BMI(kg/m ²)	14.47	30.15	20.46 2.57

Note: BMI = body mass index; * = signifies statistical significant difference between males and females

Averagely, the most frequent fingerprints patterns in the entire 10 hand digits were loops (58.4%, range = 45 - 75%), followed by whorls (27.9%, range = 16.5 - 37.8%) and arches (13.7%, range = 7.8 -

21.8%). A similar distribution of print patterns was also observed when data were categorized based on gender (Table.2).

Table 2: Distribution of Fingerprint Patterns of all The 10 Digits among Students of Faculty of Basic Medical Sciences, Bayero University, Kano.

Finger pattern	Loop(L) count (%)	Whorl(W) count (%)	Arch(A) count (%)	Males L/W/A	Females L/W/A
Right Thumb	197 (49.3)	123 (30.8)	80 (20)	114/67/36	83/56/44
Left Thumb	180 (45.0)	133 (33.3)	87 (21.8)	99/70/48	81/63/39
Right Index	207 (51.8)	126 (31.5)	67 (16.8)	116/73/28	81/54/48
Left Index	204 (51.0)	116 (29.0)	80 (20.0)	123/62/32	91/53/39
Right Middle	255 (63.8)	101 (25.3)	44 (11)	142/57/18	113/44/26
Left Middle	260 (65)	90 (22.5)	50 (12.5)	152/48/17	108/42/33
Right Ring	213 (53.3)	151 (37.8)	36 (9)	120/82/15	93/69/21
Left Ring	222 (55.5)	147 (36.7)	31 (7.8)	124/83/10	98/64/21
Right Little	300 (75)	66 (16.5)	34 (8.5)	169/35/13	131/31/21
Left Little	297 (74.3)	66 (16.5)	37 (9.3)	164/36/17	133/30/20
Overall total	2335 (58.4)	1119 (27.9)	546 (13.7)		

Note: L/W/A are the count for Loop, Whorl and Arch

The evaluation of the relationship between the fingerprint pattern and body mass index (BMI) was carried out and presented in Table 3. The distribution of the loop fingerprint patterns on the middle left ($p = 0.001$) and middle right ($p = 0.001$)

finger between persons with normal body mass index (BMI) and those with other body mass index (Obese, overweight and underweight) were statistically significant (Table 3).

Table 3: The association between body mass index and fingerprint pattern in all ten fingers

		Under weight		Over weight		Pearson's test	P
		Normal		Obese			
Little left	Loop	68	198	22	9	3.71	0.69
	Whorl	13	43	8	1		
	Arch	7	28	1	1		
Little right	Loop	69	201	22	8	0.12	0.94
	Whorl	14	45	6	1		
	Arch	5	23	3	2		
Index right	Loop	88	269	31	11	Not Computed	Not Computed
	Whorl						
	Arch						
Index left	Loop	46	141	12	5	1.95	0.37
	Whorl	30	73	11	3		
	Arch	12	55	8	3		
Thumb right	Loop	48	128	14	3	0.43	0.81
	Whorl	26	84	10	5		
	Arch	14	57	7	3		
Thumb left	Loop	46	118	13	3	1.31	0.52
	Whorl	28	88	11	5		

Ring right	Arch	14	63	7	3	0.25	0.88
	Loop	46	143	19	4		
	Whorl	34	103	10	4		
Ring left	Arch	8	23	2	3	1.33	0.52
	Loop	47	154	14	6		
	Whorl	35	94	15	3		
Middle right	Arch	6	21	2	2	14.95	0.001*
	Loop	49	188	14	3		
	Whorl	29	57	11	4		
Middle left	Arch	10	24	6	4	13.89	0.001*
	Loop	48	188	14	3		
	Whorl	31	57	11	4		
	Arch	9	24	6	4		

Note: A Perason's Chi-square analysis was done after recoding the table into a 2 (abnormal and Normal BMI) by 3 table (loop, whorl and arch). This was because there were few responders with the abnormal types of BMI, and thus combining the entire abnormal BMI will increase the power to identify a difference if it exist. *P =statistically significant ($P \leq 0.05$).

In this study, the evaluation for an association between categories of BMI and the categories of fingerprint pattern was carried out along gender lines. The significant association between the higher distribution of the loop fingerprint patterns on the middle left ($p = 0.002$) and middle right ($p = 0.002$) finger between persons with normal body mass index (BMI) remained significant among females only and not males (Table 4 and 5).

Table 4: Association of body mass index and fingerprint pattern in all fingers of male students of the faculty of basic medical sciences of Bayero University Kano

		Under weight	Normal	Over weight	Obese	Pearson Chi-square	P
Little left	Loop	32	122	9	1	2.66	0.27
	Whorl	8	23	5	0		
	Arch	3	15	0	0		
Little right	Loop	34	124	11	1	0.93	0.63
	Whorl	7	25	3	0		
	Arch	2	11	0	0		
Index right	Loop	43	160	14	1	Not Computed	Not Computed
	Whorl						
	Arch						
Index left	Loop	23	94	6	1	2.77	0.25
	Whorl	15	40	6	0		
	Arch	5	26	2	0		
Thumb right	Loop	21	83	6	1	0.79	0.67
	Whorl	15	48	6	0		
	Arch	7	29	2	0		
Thumb left	Loop	23	68	8	0	2.69	0.26
	Whorl	13	53	4	1		
	Arch	7	39	2	0		
Ring right	Loop	23	86	11	1	1.73	0.42
	Whorl	18	61	3	0		
	Arch	2	13	0	0		
Ring left	Loop	24	93	6	1	0.82	0.67
	Whorl	17	59	8	0		

Middle right	Arch	2	8	0	0	3.03	0.22
	Loop	26	110	7	0		
	Whorl	15	37	5	0		
Middle left	Arch	2	13	2	1	3.96	1.38
	Loop	25	110	7	0		
	Whorl	16	37	5	0		
	Arch	2	13	2	1		

Note: A Perason's Chi-square analysis was done after recoding the table into a 2 (abnormal and Normal BMI) by 3 table (loop, whorl and arch). This was because there were few responders with the abnormal types of BMI, and thus combining the entire abnormal BMI will increase the power to identify a difference if it exist. *P =statistically significant ($P \leq 0.05$)

Table 5: Association of body mass index and fingerprint pattern in all fingers of female students of the faculty of basic medical sciences of Bayero University Kano

		Under weight	Normal	Over weight	Obese	Pearson's Chi-square	P
Little left	Loop	35	76	13	8	1.91	0.95
	Whorl	5	20	3	1		
	Arch	4	12	1	1		
Little right	Loop	34	77	11	7	1.99	0.37
	Whorl	7	20	3	1		
	Arch	3	11	3	2		
Index right	Loop	44	108	17	10	Not Computed	Not Computed
	Whorl						
	Arch						
Index left	Loop	22	46	6	4	0.45	0.80
	Whorl	15	33	5	3		
	Arch	7	29	6	3		
Thumb right	Loop	26	45	8	2	1.79	0.41
	Whorl	11	36	4	5		
	Arch	7	27	5	3		
Thumb left	Loop	22	50	5	3	0.31	0.86
	Whorl	15	35	7	4		
	Arch	7	23	5	3		
Ring right	Loop	23	57	8	3	1.63	0.44
	Whorl	15	42	7	4		
	Arch	6	9	2	3		
Ring left	Loop	23	61	8	5	0.89	0.64
	Whorl	17	35	7	3		
	Arch	4	12	2	2		
Middle right	Loop	23	78	7	3	12.17	0.002
	Whorl	13	20	6	4		
	Arch	8	11	4	3		
Middle left	Loop	23	78	7	3	12.10	0.002
	Whorl	14	20	6	4		
	Arch	7	11	4	3		

Note: A Perason's Chi-square analysis was done after recoding the table into a 2 (abnormal and Normal BMI) by 3 table (loop, whorl and arch). This was because there were few responders with the abnormal types of BMI, and thus combining the entire abnormal BMI will increase the power to identify a difference if it exist. *P =statistically significant ($P \leq 0.05$)

Discussion

The study population in the current study had a mean height of 168 cm, mean age of 21.86 years, mean weight of 58.17kg as well as a mean body mass index (BMI) of 20.72kg/m². Thus suggesting that most of the study participants were young adults with normal BMI. The anthropometric measurements for the height and weight were higher among the male participants compared to the females and the difference was statistically significant. Male study participants were significantly older than the female participants. The distribution of the loop fingerprint patterns on the left and right middle fingers were significantly different among participants with normal BMI compared to those with Abnormal (obese, overweight, underweight) BMI. This distribution pattern was maintained among females participants when the evaluation was done based on gender. The distribution of the whorl fingerprint pattern among the participants with abnormal BMI (and not normal BMI) was also different, and the difference was statistically significant. A search of the literature revealed that few studies evaluated the association between fingerprint patterns and BMI.

Far more ulnar loops in the second and third finger on the left hand were reported among overweight and obese participants in an earlier study [32]. Both radial and ulna loop were identified to be more abundant among overweight and obese participants in the third and fourth left finger while only radial loop was reported to be more abundant in the fingerprint pattern of the left fifth finger of overweight participants while radial loop was more abundant among participants that were obese [32]. The distribution of the fingerprint pattern among the study participants in an earlier study revealed that overweight participants had a more radial loop pattern while the obese participants had the ulna loop pattern in the right-hand finger numbers 1, 4, and 5 respectively. The overweight and obese groups of participants had a higher frequency of the ulnar loop pattern in the righthand finger number 3 in this earlier study [32]. Thus, the high proportion of loop fingerprint patterns among participants with normal weight in this current study was in contrast to that of the earlier study. Also, the frequency of whorl fingerprint patterns was more abundant among

participants in the obese BMI category. The characterization of the loop fingerprint pattern as either radial loop or ulnar loop as well as the fact that the sample size evaluated in the current study was small may contribute to the disparity in study. Other studies also identified an association of obese BMI categories with arch fingerprint patterns in the fingers[28,31]. Thus, putatively suggesting that the association between fingerprint patterns and BMI are very varied from study to study emphasizing the need for a standardized methodology regarding sampling, data capturing, and analysis.

Sexual dimorphism in dermatoglyphics had earlier been reported in the literature [29]. Ulnar ridge density of males (10.01 ± 1.31) was fewer than those of the females (10.91 ± 1.69) in the right thumb[29]. Although in the current study, only evaluations of association between the fingerprint pattern (and not ridge density) and BMI was carried out, the sexual dimorphism observed is in keeping with the sexual dimorphism features reported in the literature[29]. In addition, specific evaluation of the association between BMI and fingerprint pattern revealed that there was sexual dimorphism in a different population[33,34]. Thus, the literature reports for sexual dimorphism are in keeping with the current finding.

Conclusion

The current study suggests that Fingerprint patterns can predict the body mass index of an individual especially if the participant is a female. Specifically, females with whorl fingerprint patterns in the right and left middle fingertips were more likely to be obese while those with loop fingerprint patterns in the same fingertips were more likely to be of normal BMI.

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Prediction of Age and Sex using Anthropometric Parameters among Primary Schools Children in Kumbotso Local Government Area of Kano State, Nigeria

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Abstract

There is a dearth of literature on the role of anthropometric parameters in the prediction of age and gender among school children in some populations. The current study was designed to determine the association and relationship of anthropometric parameters (height, weight (W), body mass index (BMI), Neck circumference (NC), Mid upper arm circumference (MUAC), Waist circumference (WC), Head circumference (HC), foot length (FL), hand length (HL)) to age and gender among school children in Nigeria. The design was a cross-sectional and the data was collected from 420 children whom were recruited by multistage simple random sampling. There were 211 males and 209 females. Height ($r = 0.809$), weight ($r = 0.683$), BMI ($r = 0.285$), NC ($r = 0.396$), MUAC ($r = 0.439$), WC ($r = 0.541$), HC ($r = 0.309$), RFL ($r = 0.655$), LFL ($r = 0.662$), RHL ($r = 0.723$) and LHL ($r = 0.729$) were significantly correlated with age ($P \leq 0.0001$). The measurement values for WC, MUAC, BMI and W were higher among males while the measurement values for HC, FL, HL and NC were higher among females. The differences in the measurement values were not statistically significant except the value for HC. In the adjusted statistical evaluation for the predictors of age and gender only H and left FL were for age while NC, WC and HC were for gender. In conclusion, key anthropometric measurements such as height and LFL are good predictors of age while NC, HC, and WC predicted gender among school children.

Keywords: Predictors, Age, Gender, Anthropometry, Children

Introduction

Anthropometry refers to the systematic measurements of the physical properties of humans.^{1,2} It has been used for identification of human physical variation and to correlate it with racial as well as psychological traits.¹ It helps in the understanding of anatomic structures, and forms the technique for expressing quantitative form of the human body and skeleton, it can also be regarded as the basis of ergonomics, which has become increasingly important because of its ability to prevent injuries and increase comfort.³ Anthropometrists have identified several factors, such as; age, race, gender and occupation that seem to influence human size and shape.⁴ Human skull, hands and foot measurements show some degree of interrelationship in sizes which are apparent to the human eyes and often have some correlation pattern with other biological features such as; age.⁵ The use of anthropometric measurements to estimate a

person's age and gender is not new, and can be useful for clinical, forensic as well as textile yield purposes.^{3, 6, 7} Anthropometric measures had been associated with gender as well as age among adolescents, adults and elderly in different countries.^{8,9} There are many methods that have been used to estimate individual age, but these methods are riddled with either low sensitivity or require highly invasive approaches and / or skilled experts.¹⁰⁻¹² There are other methods that putatively evaluate for age and gender through processes that incorporate the principle of anthropometrics with varying results that suggest a role for environmental factors as well as racial differences.^{4, 5, 8, 13-20} There are no studies using anthropometric measurements for the prediction of age and gender among school children in Kumbotso local government area. Determining age and gender can be of paramount importance in forensic settings where identification

is a central pillar and it can also be of importance where objective biological pointers to age and gender are needed. The study aimed at determining the age and gender using some anthropometric measurements among primary school children in Kumbotso Local Government Area (KBT L.G.A) of Kano state of Nigeria.

Materials and Methods

Permission and Consent of Study Participants

An introductory letter was obtained from Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano as well as a detailed information sheet, and both documents were presented to various Head Masters/Mistresses of the various participating primary schools in order to get permission and / or consent of the parents as well as the school authorities for the study to be carried out.

Materials

The equipment used in the study included; Weighing scale (Verma Brothers, India), Non elastic tape (Puneet Monga, India), Ruler (Ningbo Becol Stationery & Gifts Co. Ltd, China), HB pencil (GO HUA, china) and pro forma.

Inclusion and Exclusion Criteria

The subjects must be **private and / or public schools children Within Kumbotso local government area of Kano state Nigeria** between the ages of 6 to 12 years and without any physical deformity or malformation. Students that were not within the targeted age range, those whose parents declined consent as well as those with physical malformations **and those from other local governments were also excluded.**

Study Design

The study was a cross sectional study. Simple random sampling (SRS) technique was used in determining the schools to recruit study participants from and consecutive sampling was used to recruit the study participants. The study sample size was estimated using the Lwanga and Lemeshow, (1991) formula to arrive at 384 participants and 54 additional participants were recruited to attenuate the effect of any drop-out from the study. Thus, data was collected from a total of 420 students consisting of two hundred and eleven (211) males and two hundred and nine (209) females, within a period of

four weeks at two (2) public schools (Chiranchi Special Primary School and Community Based School) and 3 private schools (Grammar College Kano, Professor MKM Galadanci School and Al-Ansar International Academy). There were x males and y females from the public schools and there were z males and v females from the private schools. The recruitment of the study participants was carried out in two stages, the first stage was characterized by the teacher identifying the students whose parents had given a verbal consent. The identified study participants were then examined and asked relevant questions such as how old they were and which class they to ensure strict compliance towards inclusion and exclusion criteria of the study.

The height was measured, as a vertical distance from the sole of the foot to the vertex of the head using tape that was held in place. A ruler was used to identify the right mark corresponding to the height while the subject is standing in an upright position.²¹ The weight was measured when the participant was wearing light clothes and standing bare footed on an analog weighing scale.²² Body mass index (BMI) was calculated as the ratio of weight in kg to height in m² ($BMI = \text{kg/m}^2$).²³ Various circumferential measurements were carried out with standard techniques; mid upper arm circumference (mid-point of non-dominant arm, between the acromial process and the tip of the olecranon), neck circumference (tape around the neck), occipitofrontal circumference (occipute of the skull to the anterior portion of the frontal bone).²⁴ Waist circumference was measured in centimeters (cm) using non-elastic tape, at the narrowest region of the waist which is located between the lumbar and sacral region posteriorly and around 2 – 3 cm below the umbilicus anteriorly.^{4, 25} Hand length was measured as the distance between the middle of a line linking the styloid process of forearm bones which passed through the distal crease of the wrist joint and the tip of the middle finger while foot length was the distance between the most projecting point of the heel and end of the first or second toe (depending on which is longer) and they were both measured using a measuring tape.^{8, 27}

Statistical analysis

The mean and standard deviation for the ages of the study participants as well as those for the quantitative anthropometric parameters obtained from them were evaluated. Independent *t*-test was carried out to detect the mean difference in age and the specific anthropometric measures between gender. Pearson correlation was performed to find the relationship or influence of the anthropometric parameters (height, weight, body mass index, NC, MUAC, WC, HC, RFL, LFL, RHL and LHL) on age. Linear regression was used to predict age from the independent variables (height, weight, BMI, NC, MUAC, WC, HC, RFL, LFL, RHL and LHL). Binary logistic regression was used to determine how the dependent variable (gender) can be predicted by the

independent variables (height, weight, BMI, NC, MUAC, WC, HC, RFL, LFL, RHL and LHL). The data obtained was analysed using statistical package for service solution (SPSS) software version 25.0 and the significance level was taken as $P \leq 0.05$.

Results

Descriptive statistics

The mean $\pm$ SD for age (9.00 ± 2.00 years), height (127.79 ± 10.56 cm), weight (23.83 ± 6.17 kg), BMI (14.40 ± 2.16 kg/m²), NC (26.12 ± 1.73 cm), MUAC (17.15 ± 1.86 cm), WC (56.61 ± 5.26 cm), HC (52.10 ± 2.03 cm), RFL (20.93 ± 2.19 cm), LFL (21.31 ± 2.19 cm), RHL (14.60 ± 1.29 cm) and LHL (15.00 ± 1.31 cm) were determined in the current study as shown in Table 1.

Table 1: Mean Frequency Distribution for Anthropometric Measurements among Primary School Children in Kumbotso Local Government Area of Kano State of Nigeria

Variables	Mean $\pm$ SD	Maximum	Minimum
Age (years)	9.00 ± 2.00	12.00	6.00
Height (cm)	127.83 ± 10.56	153.00	100.00
Weight (cm)	23.83 ± 6.17	52.00	12.00
BMI (kg/m ²)	14.40 ± 2.16	26.23	8.77
NC (cm)	26.12 ± 1.73	34.90	22.00
MUAC (cm)	17.15 ± 1.86	27.00	13.80
WC (cm)	56.61 ± 5.26	80.00	36.00
HC (cm)	52.10 ± 2.03	57.20	37.50
RFL (cm)	20.93 ± 2.19	28.90	16.00
LFL (cm)	21.31 ± 2.19	29.30	16.20
RHL (cm)	14.60 ± 1.29	18.40	11.20
LHL (cm)	15.00 ± 1.31	19.00	11.40

BMI=Body Mass Index, NC=Neck Circumference, MUAC=Mid Upper Arm Circumference, WC=Waist Circumference, HC=Head Circumference, RFL=Right Foot Length, LFL=Left Foot Length, RHL=Right Hand Length, LHL=Left Foot Length,

Assessment of the distribution of Age and Anthropometric parameters based on gender

The evaluation of the distribution of age and anthropometric parameters (Height, weight, BMI,

NC, MUAC, WC, HC, RFL, LFL, RHL and LHL) based on the gender of participants was observed to be statistically significant only for the head circumference ($P = 0.0001$). Specifically, the head circumference measurement among the male participants was 51.56 ± 1.55 cm which was significantly lower than the head circumference of the female participants (52.64 ± 2.30 cm) (Table 2).

Table 2: Distribution of Age and Anthropometric parameters based on gender among primary school children in Kumbotso local government area of Kano state of Nigeria

Variables	Males (n=211) Mean $\pm$ SD	Females (n=209) Mean $\pm$ SD	T	P
Age (years)	8.99 $\pm$ 2.01	9.01 $\pm$ 2.01	-0.121	0.903
Height(cm)	127.71 $\pm$ 10.01	127.86 $\pm$ 11.11	-0.144	0.885
Weight(kg)	24.10 $\pm$ 5.36	23.58 $\pm$ 6.90	0.801	0.424
BMI (kg/m ²)	14.60 $\pm$ 1.85	14.21 $\pm$ 2.42	1.855	0.064
NC (cm)	26.21 $\pm$ 1.44	26.02 $\pm$ 1.98	1.145	0.253
MUAC (cm)	17.20 $\pm$ 1.59	17.10 $\pm$ 2.10	0.520	0.603
WC (cm)	56.94 $\pm$ 3.96	56.29 $\pm$ 6.31	1.263	0.207
HC (cm)	51.56 $\pm$ 1.55	52.64 $\pm$ 2.30	-5.65	0.0001
RFL (cm)	20.91 $\pm$ 1.95	20.95 $\pm$ 2.40	-1.182	0.856
LFL (cm)	21.30 $\pm$ 1.97	21.31 $\pm$ 2.40	-0.087	0.931
RHL (cm)	14.53 $\pm$ 1.15	14.67 $\pm$ 1.42	-1.171	0.242
LHL (cm)	14.93 $\pm$ 1.18	15.08 $\pm$ 1.43	-1.170	.242

BMI=Body Mass Index, NC=Neck Circumference, MUAC=Mid Upper Arm Circumference, WC = Waist Circumference, HC=Head Circumference, RFL=Right

Foot Length, LFL=Left Foot Length, RHL= Right Hand Length, LHL=Left Foot Length,

Table 3: Correlation of Age and the Anthropometric parameters among Primary School Children of Kumbotso local government area of Kano state of Nigeria

	Age	Height	Weight	BMI	NC	MUAC	WC	HC	RFL	LFL	RHL	LHL
Age	-	.809**	.683**	.285**	.396**	.439**	.541**	.309**	.655**	.662**	.723**	.729**
Height		-	.829**	.307**	.505**	.590**	.621**	.329**	.725**	.733**	.834**	.845**
Weight			-	.763**	.660**	.817**	.769**	.297**	.661**	.670**	.735**	.743**
BMI				-	.546**	.689**	.584**	.133**	.330**	.335**	.333**	.333**
NC					-	.621**	.619**	.330**	.470**	.472**	.477**	.487**
MUAC						-	.722**	.262**	.446**	.454**	.514**	.524**
WC							-	.320**	.606**	.613**	.592**	.600**
HC								-	.185**	.187**	.324**	.328**
RFL									-	.996**	.840**	.836**
LFL										-	.845**	.843**
RHL											-	.990**
LHL												-

Assesing the correlation between age and anthropometric parameters

Pearsons bivariate correlation was used to evaluate the association between anthropometric parameters with age. There was a significant correlation between height ($p = 1.98 \times 10^{-98}$, $r = 0.809$), weight ($p = 6.15 \times 10^{-59}$, $r = 0.683$), BMI ($p = 2.56 \times 10^{-9}$, $r = 0.285$), NC ($p = 3.47 \times 10^{-17}$, $r = 0.396$), MUAC ($p = 3.27 \times 10^{-21}$, $r = 0.439$), WC ($p = 0.29 \times 10^{-33}$, $r = 0.541$) and HC ($p = 9.63 \times 10^{-11}$, $r = 0.309$) with age as shown in Table 2. In the evaluation of correlation between RFL ($p = 8.91 \times 10^{-53}$), LFL ($p = 2.29 \times 10^{-54}$), RHL ($p = 4.28 \times 10^{-69}$) and LHL ($p = 6.13 \times 10^{-71}$) with age, the result was statistically significant (Table 3). It was also observed the anthropometric parameters (height, weight, BMI, NC, MUAC, WC, HC, RFL, LFL, RHL as well as LHL) have variable level of significant correlation with each other (Table 3)

$P < 0.001$, $N = 420$. BMI= Body Mass Index, NC= neck circumference, MUAC= mid upper arm circumference, WC= waist circumference, HC=

head circumference, RFL= right foot length, LFL= left foot length, RHL= right hand length, LHL= left hand length.

Prediction of Age using anthropometric parameters

In the step wise linear regression, the anthropometric parameters (weight, BMI, neck , waist and head circumference, right and left foot length, right hand length) were excluded from the predictive model. Only height and left foot length (LFL) were incorporated into the first and second models respectively. The first model (model 1) shows that height ($B = 0.154$) was a good predictor variable, while the second model (model 2) shows that both height ($B = 0.133$) and left foot length ($B = 0.137$) were good predictor variables (Table 4). It was observed that model 1 had the highest predictive capability ($F = 790.81$, $p = 0.000$) compared to model 2 ($F = 413.10$, $p = 0.000$). Thus, the regression equation for model 1 is: "Age = $-10.623 + 0.154$ (height)" (Figure I). The coefficient of determination (R-square) indicates that 65.4% of the variance in age is accounted for by height (Table 4).

Table 4: The step wise linear regression of anthropometric parameters for age among 6-12year old primary school children in Kumbotso local government area

Model	Variables	Unstandardized Coefficients		Sig.	95.0% Confidence Interval for B	
		B	Std. Error		Lower Bound	Upper Bound
1	(Constant)	-10.623	.700	.000	-11.999	-9.247
	Height	.154	.005	.000	.143	.164
2	(Constant)	-10.876	.694	.000	-12.240	-9.512
	Height	.133	.008	.000	.117	.148
	LFL	.137	.038	.000	.062	.212

LFL= Left Foot Length.

Adjusted association between anthropometric parameters and gender

Cumulative evaluation for the association of gender and the anthropometric parameters was also carried out. The percentage accuracy in classification was 68.8% for the regression model. The adjusted association between gender and the anthropometric parameters shows that neck circumference ($p = 0.018$), waist circumference ($p = 0.024$) and head

circumference provide the last figure as commented previously ($p = 0.0001$) were significant predictors of gender (Table 5). Height ($p = 0.140$), weight ($p = 0.364$), BMI ($p = 0.249$), MUAC ($p = 0.242$), HC ($p = 0.257$), RFL ($p = 0.257$), LFL ($p = 0.326$), RHL ($p = 0.989$) and LHL ($p = 0.691$), were not significant predictors of gender (Table 5).

Table 5: *Adjusted logistic regression for Gender based on Anthropometric parameters among primary school children in Kumbotso local government area*

Variables	B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Height	-.080	.054	2.177	1	.140	.923	.830	1.027
Weight	.129	.142	.823	1	.364	1.138	.861	1.505
BMI	-.262	.227	1.331	1	.249	.769	.493	1.201
NC	-.216	.092	5.553	1	.018	.806	.674	.964
MUAC	.128	.110	1.371	1	.242	1.137	.917	1.409
WC	-.082	.038	4.749	1	.029	.921	.856	.992
HC	.443	.073	36.431	1	.000	1.557	1.348	1.798
RFL	.601	.530	1.285	1	.257	1.823	.645	5.152
LFL	-.530	.539	.967	1	.326	.589	.205	1.693
RHL	.008	.584	.000	1	.989	1.008	.321	3.167
LHL	.232	.585	.158	1	.691	1.261	.401	3.967
Constant	-8.971	7.679	1.365	1	.243	.000		

BMI=Body Mass Index, NC=Neck Circumference, MUAC=Mid Upper Arm Circumference, WC=Waist Circumference, HC=Head Circumference, RFL=Right Foot Length, LFL=Left Foot Length, RHL=Right Hand Length, LHL=Left Hand Length. S.E=Standard Error, df=degree of freedom

Discussion

In the current study, head circumference among male participants was significantly lower than the head circumference of the female participants. Bivariate assessment revealed a significant correlation between some of the measured AP (height, weight, BMI, NC, MUAC, WC and HC) with age. The evaluation of association between RFL, LFL, RHL and LHL with age was also significant. In the adjusted linear regression evaluations, height was observed to be a good predictor of age while NC, WC as well as HC were good predictors of gender in the logistic regression model.

Earlier studies in Iranians revealed that head circumference was significantly larger in boys, not girls, and this was not similar to the current finding.^{27, 28} These earlier studies were large population-based studies for identifying normative value of head circumference among Egyptians and Iranians and this may be partly responsible for the difference in findings from the current study which was relatively small sized study. In addition, environmental differences between our study participants and those of the earlier studies may

partly account for the difference in findings.^{27, 28} In addition, even though measurements of HC had consistently revealed that males had a higher value compared to females, transition at the age of 10 years had been observed in large population studies.^{27, 28} The mean head circumference for girls at age 10 years was not significantly different when compared to the mean HC among boys at the same age.^{27, 28} Higher values for hip circumferences among females had been reported in children aged 7-13 years, and a trend for higher values of anthropometric measurements among females compared to males was reported in a research carried out in northern Nigeria which did not assess for HC.⁴ These findings appear to lend credence to the higher size of HC observed in females in the current study (with the mean age of about 9 years). The higher HC values in girls and not boys, within the age of 9-10 years can be explained by earlier growth spurt reported in girls within the age range.²⁷

In another study in which participants with variable ages were recruited based on nutritional status, it was reported that gender have significant correlation with weight (-0.314, $p < 0.001$) and height (-0.056, $p < 0.005$).¹⁹ This is in contrast to the finding of the current study and the probable reason for the difference in findings is putatively due to the age difference and nutritional status in study participants. Specifically, the current study reviewed young participants with mean age of 9 years regardless of their nutritional status, while the earlier study in the literature recruited both young and adult participants

based on nutritional status. All of the commonly evaluated anthropometric measures are influenced by changes in body proportions and composition and the degree of sexual maturation.²⁹

Higher hip, abdominal, waist and upper arm circumferences were reported among females and not males in the literature although statistical evaluation revealed that only the difference in the score / measurement values for hip and abdominal circumferences were statistically significant.⁴ In the current study, it was observed that WC, MUAC, BMI and weight were significantly higher among males compared to female participants. Although there are disparities in the parameters evaluated in the current study as against those evaluated in the literature, nevertheless, the parameters (WC, MUAC) that were common to both studies pointed to an putative association with gender but in different direction for each study. The fact that the literature study evaluated study participants aged between 7 to 60 years while the current study only evaluated patients aged 6 – 12 years old may be responsible for the disparity in findings.⁴

The role of hand dimension in the determination of gender had been evaluated in the earlier on reports.^{8, 30} The reports suggested that males averagely had a higher value for hand measurement such as hand length and breadth, which were usually 1cm higher than the values obtained for females.^{8, 30} In addition, it had been reported that irrespective of the age group of study participants, hand index usually has a calculated value that is above 44% in males and below 44% in females.^{8, 30} These finding were not in line with results of the current study in which the females tend to have higher hand length measurement values compared to males even though the difference in measurement value were not statistically significant. These could be due to the evaluations of AP measurement obtained from children with mean age of 9 years, and this is putatively the time in which females usually grow faster than males. These findings further buttress an earlier observation, that suggested that the use of hand dimensions in the determination of gender can only be sacrosanct if the moderating effect of aging is factored in.³¹ It appears that the association between NC and gender that was observed in the current study is a novel one. It postulates that the

bigger the NC, the more likely to be a male participant.

Bivariate assessment revealed a significant correlation between some of the measured AP (height, weight, BMI, NC, MUAC, WC, RFL, LFL, RHL and LHL and HC) with age in the current study. An earlier study which corroborated this finding reported that waist circumference in both males and females increases with aging and that the younger adults tend to experience greater gains in waist size when compared to older adults, due to greater weight gains during young adulthood.¹⁴ It was reported that estimates for NC percentile tended to be larger with increasing age and this was in tandem with the finding in the current study.³² Anthropometric parameters such as; weight, height and BMI had all been reported to have a strong correlation with age.¹⁹ Hand length has been reported to have little or no correlation with age among a population of elderly study participants.³³ The probable reason for the difference in finding between this hand length to age correlation studies in the literature and the findings in the current study may putatively be due to the age difference in study participants for the studies. Another probable reason is putatively because the current study evaluated the association between the different hand (right and left) with age while the study reported in the literature evaluated the association with hand length in general. Test result from step wise linear regression, presented the predictive variables for age and it was observed that only height and LFL were significant predictors of age. The regression equation was $\text{age} = -10.623 + 0.154 (\text{height})$ in model 1, $\text{age} = -10.623 + 0.133 (\text{height}) + 0.137 (\text{LFL})$ in model 2. These models suggested the amount of increase in Age that would be predicted by a unit increase in height as well as if height and LFL were combined in the model. For every one cm increase in height, a 0.154 increase in age was predicted in model 1. Similarly, for model 2, every one-centimeter increase in height as well as 1 cm increase in LFL suggests a 0.133 combined with 0.157 increases in age. Most earlier studies focused on evaluating the association between foot length and gestational age and were able to establish same.³⁴

Conclusion

In conclusion, it was observed that, neck, mid upper arm, waist and head circumferences as well as height,

weight, Body mass index, right foot length, left foot length, right hand length and left-hand length significantly correlated with age. Height and left foot length were found to be the predictors for age, although, height was observed to be the best predictor of age. In the prediction of gender, height, weight, BMI, MUAC, RFL, LFL, RHL as well as LHL were not significantly associated with it. NC and WC show valuable association with gender only in an adjusted statistical model. HC measurement showed a statistically significant difference in distribution based on gender classification before and after statistical adjustment and thus it is the only good predictor of gender in the current study.

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Prevalence of Norovirus Infection in Children Less than Five Years Presented with Diarrhoea Attending Selected Hospitals In Kano

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Abstract

Diarrhoea is the leading cause of mortality in children less than five years of age, resulting in as much as 801,000 global deaths yearly of which more than 40% occur in Africa. Norovirus is a single stranded, positive sense, non-enveloped RNA viruses of 30 - 40 nm in size characterized by a typical cup-shaped capsid belonging to the family *Caliciviridae*. Noroviruses are believed to be the major cause of Acute Gastroenteritis in children less than five years of age worldwide. This study was aimed at determining the prevalence of Norovirus infection in children less than five years presented with diarrhoea attending selected hospitals in Kano metropolis. A total of 73 participants were recruited for the study and stool samples were collected from each participant. The samples were assayed for Human Norovirus antigen using Human Norovirus Antigen ELISA kit. Four out of the 73 diarrheic samples were found positive for Human Norovirus antigen giving a prevalence of 5.5%. Statistical significance relationship between Norovirus infection and poor hygiene was observed with a p-value of 0.01. Norovirus was also found to be more prevalent in children within the age group 2-3 years old as well as those residing in rural settlements. This study has found Norovirus in cases of diarrhoea in the study subjects and thus shown that the virus should be considered as possible cause in cases of childhood gastroenteritis.

Keywords: *Norovirus, Diarrhea, Children under five years, Kano*

Introduction

Norovirus is a non-enveloped, single stranded, positive sense, RNA virus belonging to the kingdom *Orthornavirae*, phylum *Pisuviricota*, class *Pisoniviricete*, order *Picornavirales*, family *Caliciviridae*, genus *Norovirus* and Norwalk virus type specie. [1]

Human Norovirus, previously known as Norwalk virus, was first characterized in stool specimens collected during an epidemic of gastroenteritis in Norwalk and was attributed as the first viral agent shown to cause gastroenteritis. [2]

Diarrhoea is considered to be the second leading cause of death due to infections among children under 5 years of age worldwide [3] and it is the major

disease manifestation caused by most enteric viruses including Noroviruses. [4] The disease is usually life threatening in children. [5] According to the World Health Organization, there are 1.7 billion annual episodes of diarrhoea and 760 000 annual deaths due to diarrhoea among children under 5 years of age. [3] More than 40% of these deaths occur in Africa. [6] Diarrhoeal disease due to an entero-pathogen may be caused by bacteria, parasites or viruses, and the frequency of each entero-pathogen may vary from one region to another. [7] In developing countries, Noroviruses are listed among causes of diarrhoea, but the relative contribution to the total number of cases seems to be diluted in lieu of more prevalent diarrhoea-causing entero-pathogens. [8, 9] The main sources of this virus in children under five years are

contaminated water and foods. ^[10] The aim of this study is to determine the prevalence of Norovirus infection in children under the age of five (5) presenting with diarrhoea in the study area.

Materials and Methods

Study Design

The design was a hospital based cross sectional study design.

Study Area

The study was carried out in pediatrics ward of Murtala Muhammad Specialist Hospital and Hasiya Bayero Pediatrics Hospital in which majority of pediatrics patients were attended to and are both in Kano Metropolis that lies along latitude 11° N and longitude 8° E. ^[11]

Sample Size Determination

The sample size was calculated and determined using the formula;

$$n = \frac{Z^2 p q}{d^2}$$

Where;

n = number of samples

Z = statistic for level of confidence at 95% = 1.96

p = prevalence = 5% = 0.05 ^[11]

q = 1 - p = (1 - 0.05) = 0.95

d = allowable error of 5%, (0.05)

$$n = 73$$

Therefore a total of 73 participants were recruited for the study.

Study Population

Children (73) under five years of age presented with diarrhoea attending Murtala Muhammed Specialist Hospital and Hasiya Bayero Pediatric Hospital were selected randomly irrespective of female or male as they present themselves to the hospital.

Diarrheic children under five years of age attending Murtala Muhammed Specialist Hospital and Hasiya Bayero Paediatric Hospital whose parent(s)/guardian(s) consented to their participation in the research were included.

Children under five years of age attending Murtala Muhammad Specialist Hospital and Hasiya Bayero Paediatric Hospital not presented with diarrhoea and whose parent(s)/guardian(s) did not consent to their participation in the research were excluded.

Ethical Considerations

Ethical approval to conduct this research was obtained from the Research Ethics committee of Kano State Ministry of Health. Ethical approval with reference number: MOH/Off/797/T.I/1762. Detailed information about the study was given to the participant's parent(s)/guardian(s) and written consent to participate obtained before data and sample collection.

Data Collection

Socio-demographic data of the subjects were captured using an appropriately structured questionnaire.

Detailed information about the study was given to the subject's parent(s)/guardian(s) and consent to participate was sought before data and sample collection.

Sample Collection and Analysis

A small amount of stool sample was collected from the subjects in a universal container by their respective parents after they have been properly educated on the standards of sample collection.

Sample Processing

Particulates were removed from the stool sample by centrifugation and aliquot collected in plain containers.

Sample aliquots were stored at -20°C; freeze-thaw cycles were avoided.

Sample was assayed for Human Norovirus antigen using the Human Norovirus Antigen ELISA kit (MELSIN- CHINA) following manufacturer's protocol. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process in a 96 well strip microplate to analyze the existence or not of Human Norovirus Antigen in the sample.

Procedure

Fifty microliter (50 µl) each of positive control and negative control were added respectively to the positive and negative control wells. Sample (50 µl) was added to sample testing wells, and then 40 µl of sample diluent was added. One hundred micro liters (100 µl) of HRP-conjugate reagent was added to each well, then covered with adhesive tapes and incubated at 37 °C for 60 minutes. The wells were then washed five (5) times by aspirating each well,

then filling with 400 µl of wash solution. After the last wash, any remaining wash solution was removed by aspiration. The plates were then inverted and blotted dry against clean paper towels. Fifty micro liters (50 µl) of chromogen solution A and fifty micro liters (50 µl) of chromogen solution B were added to each well. The wells were then gently mixed and incubated for 15 minutes at 37 °C. Fifty micro liters (50 µl) of Stop Solution was then added to each well to give a yellow coloration. The optical density (OD) was read at 450nm spectrophotometrically and any OD above the cut-off value (0.15) was recorded as positive.

Statistical Analysis

Data obtained was analyzed using SPSS Statistics, version 20.0. The data was summarized using proportions and frequencies as appropriate; association between proportions was determined using chi-square test. At 95% confidence interval, p-

value of <0.05 was considered statistically significant.

Results

Of the 73 children recruited from Hasiya Bayero Pediatric Hospital and Murtala Muhammad Specialist Hospital in Kano Metropolis for this research, four (4) were positive for Human Norovirus antigen. Thus the prevalence of Norovirus infection in children less than five years of age presented with diarrhoea attending these hospitals was found to be (5.5%) as shown in Table 1.

Of the 73 children recruited for the study, 3 (4.1%) were less than one (<1) year, 7 (9.6%) were one (1) year, 18 (24.7%) were two (2) years, 13 (17.8%) were three (3) years, 12 (16.4%) were four (4) years and 20 (27.4%) were five(5) years. Of these, one participant (1.4%) was positive among two years of age while three (4.1%) were positive among participants in the 3 years of age category .

Table 1: Prevalence of Human Norovirus infection among children under five (5) years of age in the study population (n=73)

Age	Frequency (n)	Hospitals (n)	Positive (n)	Hospitals	Prevalence (%)
< 1 year	3	MMSH (2) HBPH (1)	0	MMSH (0) HBPH (0)	(0.0)
1 year	7	MMSH (5) HBPH (2)	0	MMSH (0) HBPH (0)	(0.0)
2 years	18	MMSH (13) HBPH (5)	1	MMSH (1) HBPH (0)	(1.4)
3 years	13	MMSH (6) HBPH (7)	2	MMSH (2) HBPH(1)	(2.7) (1.4)
4 years	12	MMSH (7) HBPH (5)	0	MMSH (0) HBPH (0)	(0.0)
5 years	20	MMSH (13) HBPH (7)	0	MMSH (0) HBPH (0)	(0.0)
Total	73	MMSH (46) HBPH (27)	3 1	MMSH (3) HBPH (1)	5.5
Grand Total	73	73	4	4	5.5

Keys: n- frequency, %- percentage, MMSH – Murtala Muhammad Specialist Hospital, HBPH –Hasiya Bayero Pediatrics Hospital

This research included 31 male participants (42.5%) and 42 female participants (57.5%). Children within the age group 0-1 year were 10 (13.7%), those within the age group 2-3 were 31 (42.5%) and those within the age group 4-5 were 32 (43.8%).

Among the participants 7 (9.6%) were from rural settlement and 66 (90.4%) were from urban settlement (Table 2).

Table 2: Socio-Demographic Distribution of the Study Participants

Variables	Frequency (n)	Hospitals (n)	Percentage (%)	Neg. (n)	Pos. (n)	Hospitals (n)	Prevalence (%)
Gender							
Male	31	MMSH (20) HBPH (11)	(27.4) (15.1)	30	1	MMSH (1) HBPH (0)	(1.4) (0.0)
Female	42	MMSH (26) HBPH (16)	(35.6) (21.9)	39	3	MMSH (2) HBPH (1)	(2.7) (1.4)
Total	73		(100)	69	4		(5.5)
Age Group							
0-1 year	10	MMSH (6) HBPH (4)	(8.2) (5.5)	10	0	MMSH (0) HBPH (0)	(0.0) (0.0)
2-3 years	31	MMSH (22) HBPH (9)	(30.1) (12.3)	27	4	MMSH (3) HBPH (1)	(4.1) (1.4)
4-5 years	32	MMSH (18) HBPH (14)	(24.7) (19.2)	32	0	MMSH (0) HBPH (0)	(0.0) (0.0)
Total	73		(100)	69	4		(5.5)
Settlement							
Rural	7	MMSH (5) HBPH (2)	(6.8) (2.7)	4	3	MMSH (2) HBPH (1)	(2.7) (1.4)
Urban	66	MMSH (41) HBPH (25)	(56.2) (34.2)	65	1	MMSH (0) HBPH (1)	(0) (1.4)
Total	73		(100)	69	4		(5.5)

Keys: n- frequency, %- percentage, MMSH – Murtala Muhammad Specialist Hospital, HBPH –Hasiya Bayero Pediatrics Hospital

Source of drinking water was found to be statistically significant to Norovirus infection ($p = 0.037$). Children who used tap water as their source of drinking water had higher prevalence of Norovirus infection (2.70%) as opposed to those who used borehole water (1.40%) and sachet water (1.40%) as their source of drinking water.

There was no statistical significance between diarrhoea and Norovirus infection ($p = 0.113$). There

was equal prevalence between children who frequently had diarrhoea (2.75%) and those who sometimes had diarrhoea (2.75%) as opposed to those who had diarrhoea for the first time (0%).

The children found positive with Human Norovirus may have not practice regular hygiene. This shows a statistical significance in this distribution ($p = 0.001$) (Table 3).

Table 3: Distribution of Human Norovirus in Relation to Possible Risk Factors

Factors	Positive (n, %)	Negative (n, %)	Total (n, %)	P value*
Source of Drinking Water				0.037
Tap water	2 (2.70)	8 (11.0)	10 (13.7)	
Borehole water	1 (1.40)	6 (8.20)	7 (9.6)	
Pure water	1 (1.40)	55 (75.30)	56 (76.7)	
Total	4 (5.5)	69 (94.5)	73 (100)	
Diarrhoea				0.113
Frequent	2 (2.75)	47 (64.4)	49 (67.1)	
Sometimes	2 (2.75)	9 (12.3)	11 (15.1)	
First time	0 (0)	13 (17.8)	13 (17.8)	
Total	4 (5.5)	69 (94.5)	73 (100)	
Hygiene				0.001
Regular	0 (0)	52 (71.2)	52 (71.2)	
Sporadic	4 (5.5)	17 (23.3)	21 (28.8)	
Total	4 (5.5)	69 (94.5)	73 (100)	

n= Frequency, %= Percentage, $p < 0.005$ is considered statistically significant, *Chi square test.

Discussion

In this study, Human Norovirus antigen from stool samples of children less than five years old presented with diarrhea has a prevalence of 5.5%.

Human Norovirus was detected in 4 (5.5%) out of the 73 samples collected for the study all of which were discovered to be samples from participants that sporadically observed hygiene. This implies that this virus is significantly associated with hygiene status ($p = 0.001$). The higher prevalence of Human Norovirus among participants with poor hygiene practice can be attributed to the fact that the virus has been described to be environmentally stable and persistent as described by Allen *et al.* [13] hence easily contractible from the environment.

This finding is comparable to studies in Taraba, Bauchi and Borno states where the prevalence of Norovirus was found to be 7%, 5% and 8%. [11] These studies also reported higher prevalence in children two years of age and a more predisposition of children who do not observe regular hygiene practices.

The current study reveals the dominance of the infection amongst children within the age group 2 -3 years. This finding is comparable to studies in Ibadan where Norovirus antigen was found to be

detected in 4 (8%) out of 50 diarrheic children under five years of age and found to be more prevalent in children within 31-36 months. [14] The higher prevalence of Human Norovirus in the age group 2-3 years can be attributed to the stoppage of breastfeeding among children in this age group. Breast milk is well known to be rich in immunoglobulin A (IgA) which has been described to be of importance in immunity to Norovirus infection. [15]

According to Mathijs *et al.* [16], conventional methods of sewage and water treatment have proven ineffective in the total removal and inactivation of enteric viruses; this may justify the higher prevalence in consumers of tap water. This is in conformity with reports by Ayukekbong *et al.* [17] who reported a higher prevalence of the infection in consumers of tap water.

The lack of statistical significance in our study between diarrhoea and Norovirus infection indicates that diarrhoea might not be a risk factor for infection however the reverse might be the case as it has been stated by previous researchers that Norovirus are major causes of acute gastroenteritis. [18]

Apparently the higher prevalence of Norovirus infection among rural inhabitants can be attributed to the suboptimal hygiene in these areas as this study

and many previous studies have shown statistical significant relationship between poor hygiene and Norovirus infection.

The prevalence of Norovirus observed in the present study is however much lower than those reported in many other parts of the world. The discrepancies might be due to difference in detection techniques.^[19]

Those studies used real time RT-PCR as the method of detection which has a higher sensitivity and specificity than the antigen targeted ELISA method used in the current study.^[20]

However, the prevalence reported in this study is higher compared to that reported in Libya (4.58%) in which Norovirus antigen was detected using enzyme immunoassay and almost comparable with a study in Turkey (5.2%) in which Norovirus antigen was detected using Immuno-chromatography (IC) methods.^[21]

Conclusion

This study was able to determine a 5.5% prevalence of Norovirus in children less than five years of age presenting with diarrhoea, and poor hygiene was identified as a risk factor for Norovirus infection.

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Prevalence of Zika Virus among Pregnant Women Attending Murtala Muhammad Specialist Hospital Kano

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Abstract

Zika virus is a single stranded RNA virus of the family *Flaviviridae*, genus *flavivirus*, closely related to dengue virus, yellow fever virus, and West Nile virus, primarily transmitted through the bite of an infected *Aedes* mosquito species. Zika virus infection in humans was first described in Nigeria (Africa) in 1954. The aim of this study is to determine the prevalence of Zika virus among pregnant women and to determine socio-demographic data of prevalence of Zika virus infection among pregnant women in Kano. Urine samples were collected from 90 pregnant women attending Murtala Muhammad Specialist Hospital, Kano. Zika virus antigen was detected using antigen targeting ELISA kit (MELISIN-China Zika virus Antigen ELISA kit-). The prevalence of Zika virus among pregnant women tested in the study was found to be 8.9 % of the total study participants. Statistically significant association was found between history of mosquito bite and presence of Zika virus antigen ($p=0.001$). No statistical association found between blood transfusion and presence of Zika virus antigen among the positive subject ($p=0.417$ and 0.890). Thus, this study revealed the prevalence of Zika virus infection among pregnant women in the study population and therefore surveillance among pregnant women is crucial for risk assessment and public health preparedness in the study area.

Keywords: *Zika virus, pregnant women, Murtala Muhammad Specialist Hospital, Kano*

Introduction

Zika virus is an envelope, single stranded, non segmented, positive-sense, RNA virus of the kingdom *Orthornavirae*, phylum *Kitrinoviricota*, class *Flasuviricetes*, order *Amarilloviraes*, family *Flaviviridae*, genus *flavivirus* and was first isolated in Uganda in 1947 but largely remained obscured in tropical Africa and parts of Asia (Musso and Gubler, 2016). Human infection with this virus was first described in Nigeria in 1954 (Gubler *et al.*, 2017). Majority of human infections remain asymptomatic but recent evidence showed that ZIKV infection can lead to congenital birth defects and severe neurological disorders (Kuadkitkan *et al.*, 2020).

It is a mosquito-borne flavivirus, which caused an unprecedented outbreak on islands in the pacific region in 2013/2014 but (Musso *et al.*, 2016). Other non-vector modes of Zika virus transmission include congenital (Oliveira *et al.*, 2016) perinatal (Ximenes *et al.*, 2019), sexual (Oussayef *et al.*, 2017) blood transfusion (Liu *et al.*, 2019) animal bite (Kazmi *et al.*, 2020), and laboratory exposure (Gregory *et al.*, 2017).

During outbreak symptoms of rash, fever, arthralgia, headache and neurological complications were observed (Cao-Lormeau *et al.*, 2016). Severe manifestations including Guillain-Barre syndrome in adults and microcephaly in

babies born to infected mothers were reported (Plourde and Blouch, 2016). Neither an effective treatment nor a vaccine is available for Zika virus, therefore, the public health response primarily focuses on preventing infection, particularly in pregnant women.

Although Zika virus has been known for decades, there is little contemporary data on the prevalence of the virus in Nigeria.

In light of the potential of severe congenital birth defects caused by Zika virus, surveillance among pregnant women is crucial for risk assessment and public health preparedness in Nigeria.

The aim of this study is to determine the prevalence of Zika virus in pregnant women attending antenatal clinic of Murtala Muhammad specialist hospital in Kano.

Materials and Methods

Study Design

The study was a cross-sectional study.

Study Area

This research was conducted in Murtala Muhammad specialist hospital, a secondary Health care institution and as well a referral center for a number of primary health care centers, it is located within Kano metropolis, Kano State. The state is located at the north-western Region of Nigeria laid between latitude and longitude 8 with a total land area of 20,760sq kilometer (Ado-Kurawa, 2009). Kano State borders Katsina State to the north-west, Jigawa State to the north-east, Bauchi State to the south-east and Kaduna State to the south-west.

Sample Size Determination

The sample size of this study was calculated and determined using the formula as follows (Cochran, 1977).

$n =$

n = sample size

z = statistics for level of confidence at 95% = 1.96

p = prevalence = (4%) = 0.04 (Mathe *et al.*, 2016)

q = $1-p$ ($1-0.04$) = 0.96

d = allowable error = 5% (0.05)

$n = 59$,

Study Population

Sixty-five (90) participants (pregnant women of different age groups, ethnicity, and educational status) were recruited in this study.

Inclusion and Exclusion Criteria

Inclusion Criteria compose of all pregnant women who gave informs consent during the period of study. While the exclusion criteria include all non-pregnant women and pregnant women who refuse to give consent.

Ethical Approval

Ethical clearance to conduct the research was sought from Kano state ministry of health. Participant's consent was also sought.

Sample Collection and Preservation

Urine sample was collected in 20ml sterile screw capped, leak free container which is DNase/RNase free. All particulate matter was removed by centrifugation for 15 minutes at 1000rpm. The supernatant was aliquoted to a volume of 1ml for subsequent analysis.

Samples were preserved at -20 degree Celsius.

Procedure

Manufacturer's instructions were strictly followed.

Sample Analysis

Enzyme Immunoassay was used for qualitative detection of Zika antigen in urine sample.

Test Principle

The kit uses a double-antibody sandwich enzyme linked immunosorbent one-step process to analyse the existence or not of Zika antigen in the samples. Add samples and HRP-labelled Zika antibodies to wells which are pre-coated with Zika antibody. After incubation and washing to remove the in combined enzyme, add chromogen solution A and B. The colour of the liquid will change into blue. At the effect of acid, the colour finally becomes yellow. The colour is measured spectrophotometrically at a wavelength of 450nm. The existence or not of Zika antigen in the samples is then determined by comparing the optical density (OD) of the samples to the cut off.

Protocol

- ✧ All reagents were prepared before starting the assay procedure. 50µl of positive control and negative control was added separately to the positive and negative well, 10µl of test sample was added, and 40µl of sample diluent was added to testing sample well, while nothing was added to the well for blank.
- ✧ Each well was aspirated and washed, the process was repeated four times for a total of five washes, it was washed by filling each well with wash solution. 100µl of horseradish conjugate reagent was added to each well; it was covered with an adhesive strip and incubated for 60 minute at 37.
- ✧ Remaining wash solution was removed by decanting, the plate was inverted and blotted against clean paper towels. 50µl of solution A chromogen and chromogen solution B was added to each well, it was gently mixed and incubated for 15 minute at 37.
- ✧ 50µl stop solution was added to each well, the plate was gently tapped to ensure thorough mixing. The Optical Density was read at 450nm using a micro titer plate, it was read within 15 minutes.

Statistical Analysis

Data obtained was analyzed using SPSS version 20.0 package, considering a ninety-five (95%) confidence interval and p value of less than 0.05 as significant difference, chi square was used.

Result

A total of 90 subjects (pregnant women) were assayed for the presence of Zika virus antigen, their ages ranged from 18 to 48 years. Majority of the study subjects 39(43.3%) are pregnant women within the age range of 18-28. Among the study subjects 74 (82.2%) are Hausa ethnic-group, 10(11.1%) are Fulani, 3(3.3%) are Kanuri, 2(2.2%) are Yoruba, 1(1.1%) is Nupe. All the study subjects were Nigerians from different states, with majority 60(66.7%) from Kano state, followed by

8(8.9%) from Jigawa state, 5(5.6%) from Katsina state, 3(3.3%) each from Adamawa, Zamfara and Bauchi states, 2(2.2%) each from Niger, Borno and Osun states, and 1(1.1%) each from Kaduna and Sokoto states. Lager part of the study subjects had secondary education 34(37.8%) while 23(25.6%) had tertiary education, 17(18.9%) had primary education and 16(17.8%) has no formal education as shown in (Table 1).

The prevalence of Zika virus among pregnant women recruited in the study was 8.9 % (8out of 90) of the total study participants, while 91.1% (82 out of 90) were tested negative (Table 2).

Table 3 shows Risk factors associated with Zika virus infection. Ninety pregnant women distributed according to Zika virus prevalence out of which 23 participants those with history of blood transfusion 3(13.0%) were tested positive, there is no statistical significance relationship between Zika virus infection and blood transfusion in this study ($p=0.417$).

Ninety (100%) of the participants has history of exposure to mosquito bite, 8(8.9%) were tested positive while 82(91.9%) were tested negative, there is statistical significant relationship between Zika virus infection and mosquito bite ($p=0.000$).

Positivity rate for animal bite (including monkey, rodents and dog) among participants, no participant out of 6 subjects who had been exposed to animal bite was positive, therefore there is no statistical significant relationship between Zika virus infection and exposure to animal bite ($p=0.890$) in our current study (Table 3).

Based on trimester out of 90 participants those in first trimester of pregnancy were 6 and all were tested negative, while those in second trimester of pregnancy out of 6 participants, 1(16.7%) was tested positive and 5(83.3%) were tested negative. Those in third trimester of pregnancy, out of 78 participants, 7(9%) were tested positive while 71(91%) were tested negative, there is no statistical significant relationship between Zika virus infection and trimester in this study ($p=0.596$).

Table 1: *Socio-demographic distribution of subjects by age group and ethnic group, state of origin and level of education*

Variables	Number Tested	Percentage (%)
Age		
18-28	39	43.3
29-38	33	36.7
39-48	18	20.0
Total	90	100
Ethnic group		
Hausa/Fulani	84	83.3
Kanuri	3	03.3
Yoruba	2	02.2
Nupe	1	01.1
Total	90	100
State of Origin		
Kano	60	66.7
Jigawa	8	8.9
Kaduna	1	1.1
Katsina	5	5.6
Bauchi	3	3.3
Niger	2	2.2
Zamfara	3	3.3
Adamawa	3	3.3
Osun	2	2.2
Sokoto	1	1.1
Borno	2	2.2
Total	90	
Educational Level		
Primary	17	18.9
Secondary	34	37.8
Tertiary	23	25.6
Not Education	16	17.8
Total	90	100

Table 2: *Prevalence of Zika virus infection among the study subjects*

Variables	Number Tested	Percentage (%)
Positive	8	08.9
Negative	82	91.1
Total	90	100

Table 3: Risk factors associated with Zika virus infection

Variables	Number Positive (%)	Number Negative (%)	p Value
Transfusion			
Yes	3()	20()	0.417
No	5()	62 ()	
Last Transfusion			
1 Month	0	0	0.164
2 Months	0	1	
3 Months	1	3	
1- 12 Months	0	6	
>1 Year	2	7	
Trimester			
First	0	6	0.596
Second	1	5	
Third	7	71	
Animal Bite			
Dog	0	3	0.890
Monkey	0	1	
Others	0	2	
None	8	76	
Mosquito Bite			
Yes	8	82	0.001
No	0	0	

p< 0.05 is considered significant

Discussion

Currently, the epidemiology and public health impact of Zika virus in Nigeria is not well understood. The diagnosis of Zika virus infections remains challenging due to the extensive serological cross-reactivity among members of the Flavivirus genus (WHO, 2016). Technically, we used a comprehensive antigen screening approach using a newly antigen targeting ELISA kit which is proved to be highly specific.

In this study, 90 urine samples were screened from pregnant women in Murtala Muhammad Specialist Hospital and found that 8.9% were positive for Zika virus infection. This rate is significantly higher than the 4% IgM reported in pregnant women in Plateau and Nassarawa states, north-central Nigeria by Mathé *et al.*(2016), this difference might be due to the difference in the target population as sampling in their study was performed in general population including urban and rural participants in 6 different hospitals, and a recent study reported an anti-Zika virus IgM prevalence rate of 6.2% among HIV-infected individuals in Nigeria using a commercial anti-

ZIKV IgM ELISA (Herrera *et al.*,2017). In contrast, the present study concentrates only on urban participants and in a single hospital with sample size of 90.

This finding is also higher when compared with the previous reports by (WHO, 2018) 0.83% in Yap state (USA) among pregnant women. This difference with our finding might be due to the unavailability of the vector for the virus in their study area. Findings of Liu *et al.* (2019) in Brazil which reported the prevalence of 4.89% among blood donors that was slightly lower than our finding might be due to the availability of the vector for the virus in their study area similar to the current study.

In this study, there is statistical significant association between positivity and exposure to mosquito bite (p=0.001) which shows that exposure to mosquito bite is a risk factor for acquiring Zika virus infection, which is in agreement with report of Grard *et al.*(2014).

In the current study, no statistical significant association was observed ($p > 0.05$) between positivity and the variables age, ethnic group, education level, state of origin, history of blood transfusion, exposure to animal bite and trimester of pregnancy.

In this study those participants within the age range of 18-28 and 29-38 has the highest frequency of positivity, this may be because reproductive women are higher in number in these age ranges.

Conclusion

In conclusion, this is the first study to investigate Zika virus infection among pregnant women in Murtala Muhammad Specialist Hospital. Our results demonstrate that Z Zika virus is prevalent in this population. Age, ethnic group, state of origin and educational level had no significant association with Zika virus infection in the population studied. However, there was no significant association between ZIKV infection, history of blood transfusion and animal bite.

Recommendations

A further larger-scale assessment of Zika virus infection among pregnant women in all the hospitals in Kano is important, using molecular techniques for detection of the virus nucleic acid like RT-PCR.

Since the findings of this study are limited by the small sample size of pregnant women, more extensive studies should be conducted to assess the prevalence in the general population of Kano.

There is need for personal protection which includes minimizing the risk of mosquito bites in the community and at home, covering the skin with clothing and, when that is not feasible, the use of mosquito repellent is advised.

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***Chlorella* Species with the Potential to Remediate Textile Wastewater: A Review**

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Abstract

Microalgal species have been employed in the treatment of industrial/municipal wastewater to remove and/or reduce the toxicity of various pollutants before releasing to the environment, due to their effectiveness, low-cost and availability. This paper reviews microalgal species from the genus *Chlorella* with the ability to remove toxic contaminants contained in textile wastewater based on published literature. The *Chlorella* species commonly used in the treatment of wastewater are *C. vulgaris*, *C. pyrenoidosa* and *C. sorokiniana*. These species remove and/or reduce contaminants through adsorption to cell surface by electrostatic attraction or by enzymatic action. They act as biosorbents for removal of heavy metals, toxic dyes and other compounds like nitrates, phosphates, ammonium, etc., to reduce toxicity of wastewater for safe discharge into the environment.

Keywords: *Chlorella* species, Microalgae, Remediation, Treatment, Wastewater,

Introduction

The amount of wastewater generated as a result of industrial development and other human activities is leading to considerable decrease in levels and quality of natural water in the ecosystem. Textile wastewater has been reported to contain high levels of toxic contaminants such as dyes which are designed to impart permanent colour to textile fabrics.^[1]

Microalgae are microscopic unicellular aquatic organisms that are found in fresh and marine habitats. They are known to act as biosorbents capable of removing colour and harmful substances from various wastewaters such as industrial, municipal, agricultural, livestock, etc. ^[2] With their photosynthetic nature, microalgae could serve as a better choice for dye wastewater treatment.^[3-5] Microalgae require high nutrients supply for growth and because they can grow heterotrophically and/or mixotrophically in the dark and/or in the presence of organic carbon source,^[6] wastewater may be one of the best medium for their cultivation, as such resulting in removal of contaminants and assimilation of organic compounds which are subsequently converted to cellular constituents such as fats and sugars.^[7, 8] Under heterotrophic conditions, microalgae obtain carbon from organic

compounds in their surrounding habitats, but in mixotrophic conditions carbon is sourced from both organic and inorganic compounds.^[9, 10] Both marine and freshwater microalgae have been reported to possess diverse metabolic pathways that allow them to breakdown a wide variety of contaminants such as dyes from industrial wastewater.^[11] They also display high sorption efficiency as a result of their large surface area and high binding affinity.^[12] Microalgae have been recommended as reliable sorbents for wastewater remediation due to their abundance and availability.^[13] They have been used in the removal of dyes from wastewater due to their cheap availability, fast and easy growth in simple medium, relatively high surface area and high binding affinity.^[14, 15] Microalgal treatment system for wastewater can provide several benefits to the environment including the generation of algal biomass for biofuel production, uptake of nutrients and heavy metals, utilisation of carbon dioxide to mitigate global warming as well as achieving efficient wastewater treatment.^[2, 16]

Microalgae biosorption of metabolites of contaminants (such as hydroxide, carboxylate salts, ammonia, and phosphate) and their associates that are present in wastewater as a result of their cell wall components which triggers electrostatic force of

attraction.^[17, 18] Microalgal cell walls contain the like of amino, carboxyl, sulphate, phosphate and imidazole groups associated with polysaccharides alginic acid and proteins which play an important role in binding various contaminants,^[19] while the extracellular polymeric substances in the algal cell membrane help in the adsorption of contaminants from wastewater. Microalgal production and excretion of significant amount of organic materials may also lead to the binding of heavy metals and organic contaminants on the cell surface.^[14]

With the vast amount of algal species known, only a few have been investigated for their ability to remediate wastewater. A number of the species are efficient in removing toxicants from wastewater, promising an eco-friendly and sustainable option for tertiary wastewater treatment.^[20] Microalgal treatment is more efficient, cheap and safe in removing toxicants such as synthetic dyes and heavy metals from textile wastewater.^[21, 22] Several microalgal species such as *Chlorella*, *Scenedesmus*, *Chara*, *Dunaliella* and *Oscillatoria* species have been used as adsorbents/bioaccumulators of dye from industrial wastewater.^[9, 19] This is due to their ability to grow fast and mitigate carbon dioxide in wastewater^[23] as well as produce important molecules such as fatty acids, phenolic compounds, volatile compounds, sterols, proteins, amino acids, peptides, vitamins, polysaccharides and pigments.^[24]

Microalgae have been reported to have the potential to remove colour and other contaminants from dye wastewater through several mechanisms such as assimilation, precipitation, biosorption, bioaccumulation,^[2] bioconversion and biocoagulation. This is due to their ability to utilize organic pollutants for metabolic processes,^[25, 26] and consequently leading to the production of large amount of algal biomass that can be used for biofuel production.^[7, 27, 28] Dye removal in wastewater by microalgae is dependent on dye concentration and algal biomass^[29] and is achieved when the dye molecules bind to functional groups on the algal cell surface breaking down the chromophore bonds to build cellular components.^[7] Colour removal is usually related to the number of azo bonds in the dye molecule.^[30] Some microalgal species possess azoreductase, an enzyme that breaks azo bonds in dyes at optimum conditions.^[31] Azoreductase

isolated from a green algal species decolourized two azo dyes (tartrazine and ponceas) completely.^[32] Many studies have reported the effectiveness of using azoreductase extracted from microalgae to breakdown azo dyes.^[15, 32, 33] Some of these studies include the breakdown of methylene blue by *Chlorella pyrenoidosa*, acid red 247^[34] and acid blue (290 and 324) by *Spirogyra rhizopus*,^[35, 36] triphenylmethane and malachite green dyes by *Cosmarium* sp.^[37] and the breakdown of other azo dyes into aromatic amines that were further catabolized into organic compounds by *Pithophora* sp., *Nostoc muscorum* (blue green algae), *Ulvalactuca*, *Sargassum* sp. and *Desmodesmus* sp.^[38] Waqas *et al.*^[39] reported that microalgal species utilize azo dyes to obtain carbon and nitrogen for their growth as well as remove phosphorus, heavy metals, pesticides, organic and inorganic toxins and pathogens from wastewater.^[2, 40]

Majhi *et al.*^[41] suggested that tolerance to toxic contaminants and high adsorption rate are the main factors that lead to chemical stability in microalgal decolourization. Decolorization of dye by microalgae involves three different mechanisms which involves; carbon dioxide and water; conversion of chromophore material to the non-chromophore material; and, the adsorption of chromophore onto the algal biomass.^[42]

Microalgal dye removal gradually becomes slower with time, as it depends on the availability of dye molecules or volume of the adsorbate, adsorbent and potential charge difference. Therefore, once the dye molecules are attached to the surface of the adsorbent, the freedom of movement becomes lesser due to entropy factor as well as the unavailability of surface area.^[41]

Adsorption has been shown to be one of the most promising options for removal of non-biodegradable dyes from aqueous streams using activated carbons which are the most common, versatile and very effective adsorbents for this process. Algal adsorption rate during dye removal have been reported to be higher at the beginning and declines at later stages.^[14, 43] Temperature and other influencing parameters always affect the rate of sorption, for example, increase in temperature of adsorbate increases the rate of algal adsorption^[6, 16] while pH

variability directly affects the uptake of dye and thus the adsorption process. A report by Majhi *et al.*^[41] indicated that algal species remove dye from textile wastewater at an optimal pH 7. Similarly, the effectiveness of microalgal biosorption is dependent on the functional groups contained in their cell wall.^[44]

***Chlorella* species**

Chlorella species are freshwater thallophytes rich in chlorophyll which is important in sunlight capturing for energy conversion. The *Chlorella* genus consists of spherical-shaped unicellular green microalgal sub-species found in freshwater, brackish or saline water.^[45] They are fast growing, have the potential to uptake nutrients and bind to most of the toxic heavy metals in wastewater, and also very effective in remediation of organic pollutants.^[46] Therefore, *Chlorella* species have the capacity to tolerate and survive in the presence of various organic pollutants in wastewater, gradually accumulating and eliminating them.^[2, 47, 48] Additionally, their high surface area makes them suitable for removal of dye molecules from textile wastewater.^[49] Degradation of dyes by *Chlorella* depends on their toxicity and chemical structure; the species have been reported to be capable of removing dyes with very high toxicity.^[50]

Chlorella species serve as effective adsorbents removing toxic metals and organic dyes from wastewater.^[51] They are also effective in removal of nitrogen, phosphorus, heavy metals and other harmful compounds of different concentrations from various wastewater types.^[52, 53] The species were reported by Kumar *et al.*^[54] to have successfully removed colour from textile wastewater to 43% efficacy. A report by Cheriaa *et al.*^[55] indicated that *Chlorella* species cultivated in different textile dyes had variability in the decolourization with respect to the dye type (for example, indigo had 89.3% and crystal violet, 72.5%). Yan and Pan^[56] reported that *Chlorella* species were able to metabolize more than 30 different synthetic dyes. Several *Chlorella* species like *C. vulgaris* and *C. pyrenoidosa* were reported to be capable of breaking down azo dyes to aromatic amines which are subsequently metabolized to simpler organic compounds or carbon dioxide, thus, detoxifying them.^[57] The mechanism of dye removal by *Chlorella* species is

mainly due to their biosorption potential which depends on the rate of algal biomass production.^[58] *Chlorella* species are also very important in the production of biofuels, cosmetics, food, pigments as well as bioenergy generation.^[24, 46]

Chlorella vulgaris

Chlorella vulgaris is a microalga having many structural elements similar to those in higher plants. It is fast growing, survives harsh conditions and resistant to certain invaders. It is mixotrophic and thus combines both autotrophic and heterotrophic processes to take up organic materials from wastewater during treatment.^[59] *C. vulgaris* has the ability to breakdown and remove certain compounds and nutrients (such as azo compounds and heavy metals) contained in textile wastewater, to permissible limit for discharge into the environment.^[53, 60]

Donmez *et al.*^[61] studied biosorption of heavy metals using *C. vulgaris* and *Scenedesmus obliquus* in synthetic wastewater at a high concentration up to 250 mg/L, and the optimum biosorption was seen at pH of 5.0, 4.5 and 2.0 for copper, nickel and chromium, respectively. Chromium uptake for *Chlorella sp.* and *Scenedesmus sp.* were 23% and 16%, respectively. Nezbyrsky *et al.*^[62] also reported *C. vulgaris* to have completely removed cadmium, copper and zinc that were present in wastewater.

Biosorption of three reactive dyes (remazol black B, remazol red and remazol golden yellow) with dried biomass of *C. vulgaris* investigated by Aksu and Tezer^[31] revealed highest dye uptake at pH and temperature of 2.0 and 35°C respectively in remazol black B. In another study *C. vulgaris* was reported to have bioaccumulated 75% of the dye contained in textile waste effluent.^[63] The percentage removal of textile dyes by *C. vulgaris* was reported as 50%, 63.9% and 45.7% for supranol red 3BW,^[64] blue and green dyes,^[65] respectively. The colour removal was dependent on dye concentration, period of algal inoculation and quantity of algal biomass.^[65] *C. vulgaris* was also used in the removal of reactive black 5, direct blue 71 and disperse red 1, and the optimum adsorption recorded was 200mg/L for reactive black 5, 300mg/L for direct blue 71 and disperse red 1. Percentage decolourization for all the three dyes within 10 days at temperature of 40°C

was 80%, 78% and 84% respectively.^[31] Chin *et al.*^[13] reported that *C. vulgaris* decolourized methylene blue by surface adsorption through electrostatic interaction at 84.08% within 3 days. El-Kassas and Sallam^[63] reported *C. vulgaris* to have decolourized azo dyes contained in textile wastewater to a percentage of 75.68% which lead to the reduction of its COD level to 70%. The ability of *C. vulgaris* to remove a variety of azo dyes may be attributed to the activity of azoreductase,^[66] the accumulation of dye ions on the surface of algal biopolymers and diffusion of the dye molecules from aqueous phase onto solid phase of the biopolymer.^[67]

C. vulgaris have the potential to rapidly, efficiently and effectively remove reactive dyes such as remazol red and remazol golden yellow to very low concentrations and to accumulate large amounts of dyes through adsorption.^[30] *C. vulgaris* was reported to have high tolerance to azo dyes, having the potential to grow in the presence of 400 mgL⁻¹ of a monoazo dye.^[57] The ability of *C. vulgaris* to break down the azo bond which resulted in upto 50.0% colour removal has also been observed by Lim *et al.*^[64].

The use of *C. vulgaris* in the treatment of dye wastewater is an eco-friendly and effective option which recycles nutrients and improves water quality.^[60] Many researches have shown *C. vulgaris* fixating up to 74% of carbon dioxide, absorbing 45-97% nitrogen, 28-96% phosphorus and reducing chemical oxygen demand (COD) by 61-86% from textile wastewater.^[64, 68] Some reports also indicated a complete removal of ammonium and phosphorus from wastewater.^[59] *C. vulgaris* has also been cultivated on wastewater to, in addition to removing organic pollutants, produce a high yield of biomass to generate biodiesel.^[69, 70]

Chlorella pyrenoidosa

C. pyrenoidosa is a unicellular green microalga found in fresh and marine water.^[71] It contains high protein, vitamins, minerals, dietary fibre, nucleic acids, enzymes and high chlorophyll content.^[72] It is thermophillic and medicinally used as a chelatory agent to extract toxic compounds from the body.^[73] *C. pyrenoidosa* can grow and flourish in all wastewater types, producing biomass important in

large scale biodiesel production.^[71, 74] It was used by Jinqi and Houtian^[38] to treat dye wastewater which revealed an effective result. In another study by Pathak *et al.*^[15] *C. pyrenoidosa* decolourized methylene blue above 90%. Cadmium was metabolized completely by accumulation and sorption during the treatment of textile wastewater with *C. pyrenoidosa*.^[75, 76] It was also used to remediate textile wastewater and reduced its nitrate, phosphate and organic load by 62%, 87% and 81% respectively.^[77] *C. pyrenoidosa* was also able to remove more than 80% heavy metals contained in wastewater within 15 days.^[76] Pathak *et al.*^[78] reported *C. pyrenoidosa* to be very effective in pollutant removal from dairy wastewater with a significant lipid production. Majhi *et al.*^[41] in their research on dye removal from textile wastewater using immobilized and free cells of *C. pyrenoidosa* revealed 98% and 87% percentage decolourization respectively. Therefore, *C. pyrenoidosa* has great potential in wastewater remediation and biomass generation to produce biofuel.

Chlorella sorokiniana

Chlorella sorokiniana is a thermo-tolerant, fast growing microalga capable of mixotrophic growth in various carbon sources, thus, making it ideal for cultivation on waste feed stock.^[79, 80] Lizzul *et al.*^[69] and Kotoula *et al.*^[9] reported that *C. sorokiniana* grows and flourish under both mixotrophic and heterotrophic conditions. Its optimal growth is attained at temperature range of 35-40°C,^[81] with photosynthetic doubling time as low as 4-6 hours.^[82] *C. sorokiniana* has also been reported to grow and flourish on wastewater under conditions that are unfavourable to other algal species.^[81] It is also capable of producing substances such as lipids, polysaccharides and other cellular products that could be used in bioenergy generation.^[83]

C. sorokiniana was reported to have completely removed nitrogen and reduced the levels of carbon monoxide, carbon dioxide and nitrogen oxides from wastewater within 4 days.^[80] This was achieved as a result of nutrients requirement by the algae for growth.^[84] *C. sorokiniana* has been used for treatment of raw municipal wastewater achieving partial decrease of major pollutants,^[10] as well as for the removal of organic micro-pollutants from water and wastewater.^[85, 86] Kotoula *et al.*^[9] observed that

C. sorokiniana has the ability to completely eliminate COD and partially reduce the concentrations of nitrogen and phosphorus contained in industrial/municipal wastewater under mixotrophic condition.^[87, 88] Tang *et al.*^[89] constructed a consortium of *Aspergillus* species and *C. sorokiniana* to treat dye wastewater and the result showed high efficiency in COD, nutrients as well as dye removal when compared with using individual strains of the two organisms.

Conclusion

Several researchers have reported the effectiveness of the use of various microalgal strains in the treatment of textile wastewater. In order to potentially improve wastewater treatment, microalgae such as those from the *Chlorella* genus can be effectively employed to bioremediate dyes, nutrients and contaminants contained in wastewater, so as to reduce environmental pollution. The use of *Chlorella* species is one of the safest, reliable and cost-effective methods to be employed in wastewater remediation, as it produces clean water, generate valuable products as well as provide eco-friendly energy sources such as biofuels which are readily biodegradable. *C. vulgaris*, *C. pyrenoidosa* and *C. sorokiniana* are the species that have been used in the remediation of wastewater, though; new researches are ongoing to identify other *Chlorella* species that can successfully remediate all kinds of wastewaters.

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A Comprehensive Review of the Factors Associated with Pentazocine Use and Misuse by Sickle Cell Disease Patients in Nigeria

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Abstract

Nigeria is one of the countries with the highest burden of Sickle Cell Disease (SCD) in the world. The condition is usually associated with a frequent painful crisis that may warrant prolonged treatment with analgesics. Pentazocine abuse was found to be very common among these patients and the prevalence could even be underestimated. The aim of this review is to discuss the nature and pattern of pentazocine use and misuse in patients with SCD in Nigeria, to assess factors associated with the conditions, and propose solutions to some of the problems. The study reviewed related articles, case reports and series concerning SCD patients, use and misuse of pentazocine, the prescription patterns, and factors associated with the conditions in Nigeria. Most articles were sourced from Google and Google scholar because other medical data bases like PubMed and Medline yielded few published articles. The review considers all relevant articles from January 1980 to January 2019 which were published in the English language. A lot of factors concerning the patients, families/ caregivers, healthcare team, environment, government, and also drug itself were found to be associated with the conditions. There was a high prevalence of pentazocine addiction among SCD patients in Nigeria. Easy accessibility, availability, and low cost made pentazocine very popular. Both caregivers and physicians underrate the pain, leading to mismanagement of patients. Mismanagement of chronic pain in SCD patients predisposes patients to opiates abuse. However, pentazocine availability and low cost promote its misuse.

Introduction

Drug addiction is a chronic disease that is associated with a strong desire to seek and use drug; it is compulsive and very difficult to control, despite harmful consequences. ^[1] However, addiction is a global menace and a burden that every government, families, and patients have to deal with. Although there are many types of addiction, in which drug addiction is the most popular and opioid-use disorder is the most common form of addiction in humans. ^[1] Among the opioids, pentazocine is well known for its potency and addictive nature.

Activities that promote basic life functions such as eating, sex, etc. causes feelings of pleasure. This is due to the release of a neurotransmitter called Dopamine (DA), by neurons in the ventral tegmental area (VTA) to other pleasure associated areas of the brain like the nucleus accumbens (NAc). ^[2] Other areas of the brain such as the hippocampus can associate this pleasurable feeling with a specific context and bring to mind the memory of this pleasure whenever a person encounters such context again. If left unchecked, humans would continue to

seek this pleasure by any means. However, feedback from the prefrontal cortex (PFC) help to stem and control the desire. ^[2] Most addictive drugs like heroine, oxycodone, and pentazocine are used to relieve pain; however, they can activate this natural reward process to cause intense pleasure. ^[2] Use of these drugs in the absence of significant pain can encourage repeated misuse of the drug for the pleasure only; this may later lead to tolerance and dependence. It also leads to abnormal structural changes in the brain, which compromises the PFC and its ability to regulate/ control the drive. ^[2] Because of the dependence, any attempt to quit the drug is followed by annoying withdrawal symptoms, fear of which makes abusers hooked to the drug. At first, people take drugs for pleasure and voluntarily; later, it becomes compulsive. The vicious circle continues, and until it's broken, it can continue unabated forever.

Sickle cell disease (SCD) and its variants are genetically acquired conditions that affect many systems in the body. It is caused by the substitution of thymine for adenine at the 6th codon of the beta-

chain gene, GAG to GTG which may results in the formation of valine instead of glutamate; a gel-like, insoluble form of hemoglobin (Hb) that can form Hb crystals/ tactoids especially during hypoxia or dehydration. [3] The diagnosis is usually made very early in life (6-12 months) by hemoglobin electrophoresis, but the clinical abnormalities do not usually occur before 6 months of age. The disease originated and is predominantly found in West Africa, and to a lesser extent India, and the Mediterranean region. [3] The disease is distributed equally among the sexes, with no sex predilection. [3]

There are various genotypes including the HbSS, HbSC, HbS/ hereditary persistence of fetal Hb, HbS/HbE syndrome, and HbS/b-O, or HbS/b+ thalassemia, etc. However, HbSS, the homozygous form or the sickle cell anemia (SCA) is the most common and severe form of the disease. [3] Half of the patients with HbSS experience painful vaso-occlusive crisis (VOC); however, the frequency is highly variable between patients. Common presenting complains includes but are not limited to anemia, acute/ chronic VOC, infections, growth retardation, acute chest/ bone pains, and multiple organ damage from micro infarcts. Painful VOC is the most common presenting complain and can easily be triggered by hypoxemia, dehydration, and changes in body temperature. [3] The painful VOC usually begin suddenly, and may last several hours to days; but can terminate abruptly. [3] One of the main reasons why patients seek help is pain, and opiates and opioids are used extensively by the physicians.

Pentazocine is a synthetic opioid analgesic with little or no abuse potential. [4] However, it was soon found to be associated with both psychological and physical dependence, because of its similarity with morphine. [5] It is an agonist at the kappa (κ) and sigma (σ) opioid receptors and has a weak antagonist action at the mu (μ) receptor. [6, 7] Its analgesic efficacy is 25–50% that of morphine, when administered intra-muscular (IM), and comes in both oral and parenteral formulations. [8] Main effects include euphoria, sedation, and dysphoria. [6, 9-11] Pentazocine can be used for the treatment of moderate to severe pain; as a preanesthetic medication; or as a supplement during general anesthesia. Pentazocine undergoes hepatic

metabolism, has a half-life of ≈ 12 hours and is excreted in the urine. The onset of action develops within 15 to 20 minutes following IM administration. The duration of action is approximately 3 to 5 hours. [4] Pentazocine can cause respiratory and cardiovascular depression and other side effects similar to morphine or other opioids. [12] Because of the irritation and damage to the skin as a result of subcutaneous (SC) administration, it is most preferably given IM. [12, 13] Abrupt termination of pentazocine is associated with withdrawal symptoms, including abdominal cramps, high temperature, rhinorrhea, restlessness, anxiety, and lacrimation. [7,14]

There is a lot of literature concerning addiction of SCD patients to some of the analgesics, most especially pentazocine worldwide. However, in Nigeria, there is a limited number of studies concerning addiction in general, not to talk of specific drug addictions like Pentazocine in specific conditions like SCD. Although Pentazocine-use disorder is rampant, however, little information has been reported about addiction of SCD patients to Pentazocine in Nigeria. [15,16] With the limited studies on the addiction of SCD patients to any of the analgesics, the prevalence was found to be higher than expected. [17] However, a review concerning nature, pattern, and factors associated with Pentazocine addiction in SCD patients is grossly lacking. The objectives of this review are therefore to discuss the nature and pattern of pentazocine use and misuse in patients with SCD in Nigeria, to assess factors associated with the conditions, and propose solutions to some of the problems.

Method

The study is a narrative review of case reports and case series of studies conducted on SCD patients in Nigeria who were addicted to analgesics, specifically pentazocine. Most articles were sourced from Google and Google scholar (46 articles). Other medical data bases like PubMed, Medline, CINAHL, and Cochrane. The main keywords considered in this review were pentazocine use and misuse/abuse/addiction, Sickle cell disease patients, Nigeria, opiates/ opioids, and analgesics. The available, online-published articles written in English language related to the above key words from January 1980 to January 2019 were included in

this review. Among the 46 articles reviewed, 9 were case reports, 1 was a case review, and others were relevant articles. Some results were expressed as means $\pm$ SEM, tables, figures, bar charts and geographical maps. Microsoft Office Excel version 2007 and SPSS version 15.0 software were employed in analyzing the data.

Results

A study on SCD patients found an overall prevalence of opiate dependence to be 17.8%, out of which 53.8% were dependent on Pentazocine.^[18] The prevalence of pentazocine addiction in SCD patients in Nigeria was found to be much higher than that obtained in other parts of the world.^[17] The prevalence was also reported to vary based on the three major regions of Nigeria (North, South, and Western Nigeria), with the highest prevalence found in the north.^[19] However, highest number of cases were reported from the southern part of the country (Figure 1).

The age range of SCD patients who were addicted to Pentazocine starts from as young as 13 years^[17] to

as old as 68 years.^[20] Majority of the cases were between the age ranges of 25 to 54 years (Figure 2). Both males and females were equally affected by the condition (Table 1). However, in terms of the dose, males averagely inject about 7.5 ampoules of pentazocine (228 mg) daily for an average of 3.7 years, when compared to females (133 mg, $\approx$ 4.5 ampoules/ day for 2.6 years) (Table 1).

Most of the patients' lives with both their parents (male 20%, female 15%); however, some were under the care of only their mothers (male 10%, female 20%) (Figure 3). All the ladies were not married; however, 5% of the men were able to marry (Figure 3). More women (10%) lives alone than men (5%) (Figure 3). All the patients were totally dependent on their family/ caregivers for the drug expenses, except one person who is gainfully employed (Table 2). The highest amount spent by a patient was about ₦270,000 (\$750) per month (Table 2). A summary of the various factors that are associated with pentazocine use and misuse by the SCD patients were enumerated in Table 3.

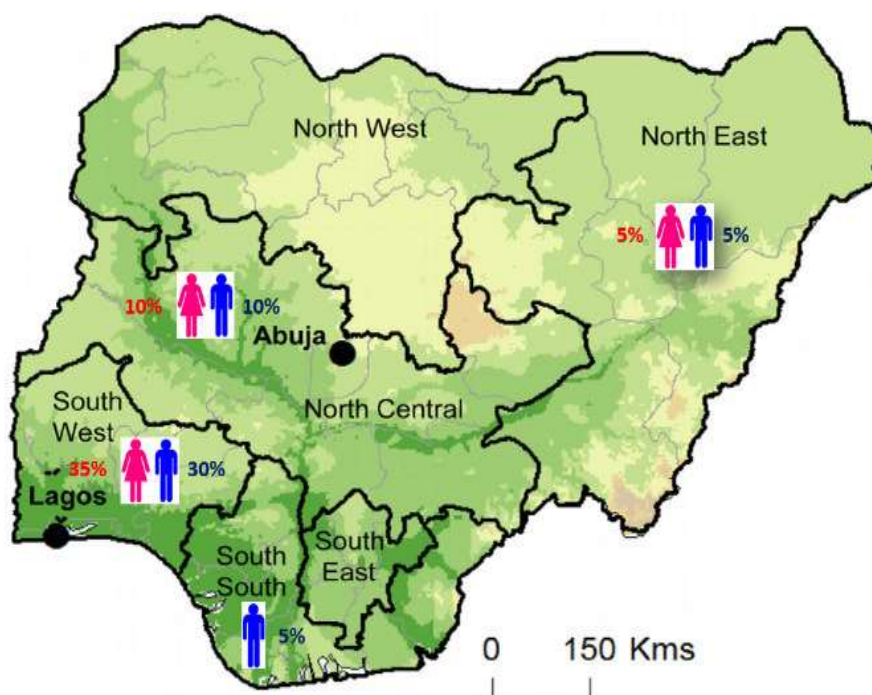


Figure 1: Distribution of the reported cases of pentazocine addiction in SCD patients across the six geopolitical zones of Nigeria.

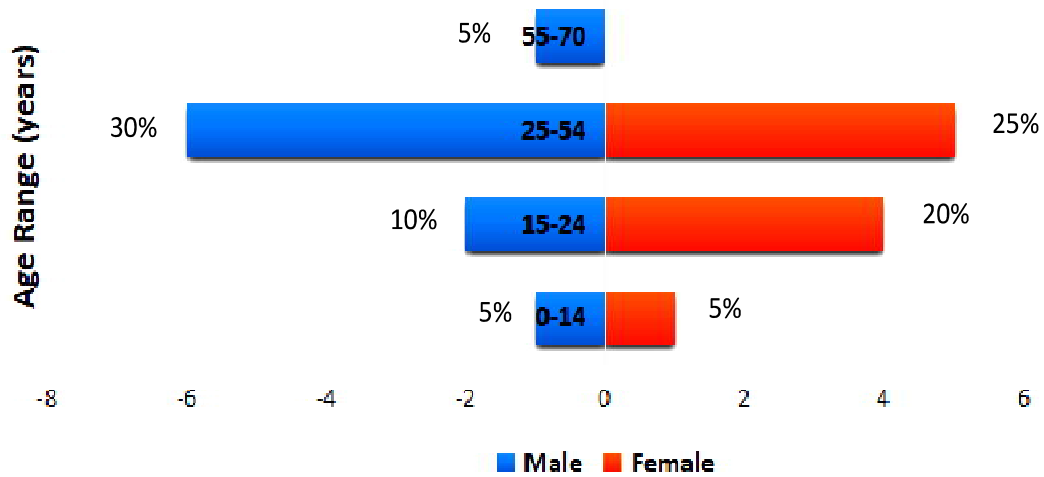


Figure 2: Age distribution according to the gender of sickle cell disease patients who were addicted to pentazocine.

Table 1: Average Daily Dose and Duration of Exposure to Pentazocine Based on the Gender

Gender	No. of reported cases of pentazocine abuse	Mean of the maximum daily dose	Mean Duration of Abuse (years)
Male	10	228 mg ($\approx$ 7.5 ampoules)	3.7
Female	10	133 mg ($\approx$ 4.5 ampoules)	2.6

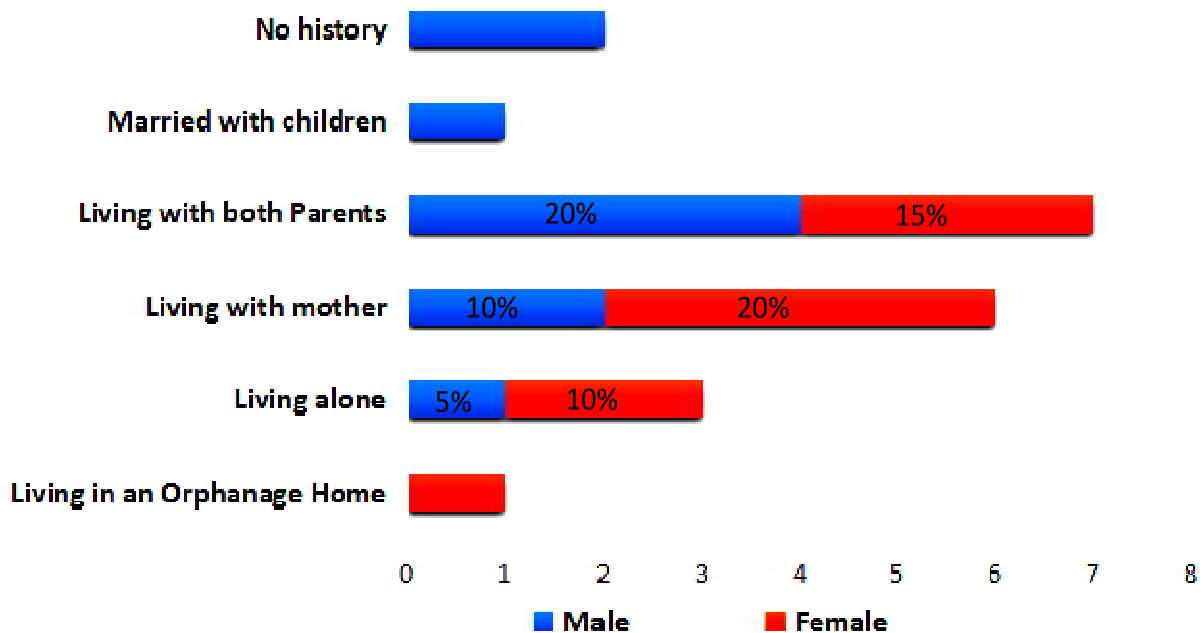


Figure 3: Living status of sickle cell disease patients who are addicted to pentazocine.

Table 2: Educational Background, Employment and Financial Status of SCD patients who are addicted to pentazocine.

Age (years)	Sex	Educational status	Employment status	Income/ month	Daily expenses on Drugs
34	M	Degree	Unemployed	Nil	Not reported
28	M	Degree	Civil servant	₦42,000 (\$300)	Not reported
24	F	Undergraduate	Student	Nil	Minimum of ₦800 (\$5)
17	F	Secondary school certificate (S.S.C.)	Secondary school graduate	Nil	₦1,000-₦4,000 (\$5-\$17)
30	M	S.S.C	Unemployed	Nil	Not reported
25	F	Not reported	Not reported	Not reported	Not reported
14	F	Not reported	Not reported	Not reported	Not reported
20	M	Not reported	Not reported	Not reported	Not reported
21	M	Not reported	Not reported	Not reported	Not reported
40	F	Not reported	Not reported	Not reported	Not reported
18	F	Not reported	Not reported	Not reported	Not reported
29	F	Not reported	Not reported	Not reported	Not reported
53	M	Not reported	Not reported	Not reported	Not reported
13	M	Not reported	Not reported	Not reported	Not reported
28	M	Not reported	Not reported	Not reported	Not reported
26	F	Not reported	Not reported	Not reported	Not reported
40	F	Graduate	Unemployed	Nil	₦1000-₦9000 (\$3-\$25)
23	F	Undergraduate	Student	Nil	₦600 (\$4)
25	M	University dropout	Unemployed	Nil	₦300 (\$2)
68	M	Pharmacy Technician	Retired dispensary attendant	Not reported	Not reported

Table 3: A Summary of Factors Associated with the Use and Misuse of Pentazocine Among SCD patients in Nigeria.

Category of the Factors	S/ No	Problems	Gravity of the problem	References
Environmental factors	1	Pentazocine not considered as controlled drug	Severe	[15, 16, 18, 21, 22]
	2	Pentazocine easily available without Doctor's prescription	Severe	[16, 18, 22, 23]
Government associated factors	1	Laxity and failure of the government to enforce laws that regulate drug availability and accessibility.	Moderate	[16, 23]
	2	lack of proper rehabilitation programs	Mild	[22]
	3	lack of standard protocols	Mild	[22]
	4	lack of standard guidelines on the use of analgesics.	Mild	
	5	Various specialists are lacking	Mild	
	6	Maldistribution of the few specialist	Mild	[16]
Factors Related to the Drug	1	High potency of pentazocine to relieve patients' pains, and cause euphoria and sedation.	Severe	[22, 23, 24]
	2	Pentazocine was cheap drug compared to other analgesics.	Severe	[16, 22, 23]
	3	Oral pentazocine was not available	Mild	[15]
	4	Pentazocine was associated with high risk of addiction	Mild	[16]

Factors Related to the Healthcare Team and the Hospital Environment	5	Pentazocine causes high psychological dependence	Mild	[18]
	1	Physicians' gross and inappropriate drug prescriptions to patients with SCD	Mild	[25]
	2	Narcotic analgesics were the most prescribed drugs to SCD patients	Mild	[25]
	3	Availability of the drug shapes Doctors' choice of the prescribed analgesic.	Mild	[16]
	4	Pentazocine was a favorite drug to many physicians in Nigeria.	Mild	[23]
	5	Choice of pentazocine was related to its potency, availability, and affordability.	Mild	[23]
	6	Several exposures to pentazocine before puberty.	Mild	[23]
	7	Physicians' prescription of strong analgesics to calm down patients	Mild	[23]
	8	Prolonged treatment with pentazocine	Mild	[23]
	9	Health workers' distorted perceptions of SCD patients toward analgesics	Mild	[23]
	10	SCD patients were considered "drug seekers"	Mild	[22]
	11	Suboptimal treatment of pain in SCD patients	Mild	[22]
	12	Physicians underate the severity of their patients' pain	Mild	[22]
	13	Physicians' heightened fear of addiction	Mild	[22]
	14	Nurses' gross carelessness, mismanagement, and poor attitudes towards patients	Mild	[17]
	15	Generalized mistrust between SCD patients and their healthcare team	Mild	[17]
	16	Some nurses ignore their patients complains or refuse to serve medications as and at when due.	Mild	[17]
	17	Some patients prescribe and forge Doctors' signatures in order to obtain pentazocine.	Mild	[21]
	18	Health care workers do not protect the identity of drugs	Mild	[23]
	19	Some Doctors' fail to identify early signs of drug misuse till complications arise.	Mild	[20]
	20	Assessment of pain score not frequently performed.	Mild	[19]
	21	Failure of physicians to educate their patients and care givers concerning SCD and addiction.	Mild	[19]
	22	Poor documentation of pentazocine abuse	Mild	[23]
	23	Some physicians had difficulty differentiating genuine from fake pain complaints.	Mild	[23]
	24	Some general practitioners were inexperience in the management of SCD patients.	Mild	[23]
	25	Lack or poor adherence to the standard pain management protocols	Mild	[23]
	26	Lack of holistic approach in the management of painful crisis.	Moderate	[15, 26]
	27	Inadequate specialist	Mild	[17]
	28	Other pain treatment options not explored.	Mild	[16]
	29	Pentazocine addiction underreported	Moderate	[15, 16]
Factors Related to the Patients, their	1	Frequent painful crisis was associated with chronic exposure to opiates and addiction	Mild	[16]

Families/ Caregivers and Friends	2	Most patients exaggerate their pains	Mild	[23]
	3	Most addicted patients equally had greater disease severity	Mild	[24]
	4	Caregivers underrate the patients' intensity of pain.	Mild	[22]
	5	Prolonged exposure to pentazocine lead to addiction.	Severe	[16, 22, 23]
	6	Patients' emotional states usually ignored in the treatment.	Mild	[27]
	7	Intense craving and fear of withdrawal symptoms maintains the addictive behavior.	Severe	[19, 21-23, 26, 28]
	8	Repeated use of unsterilized needles predisposes the addicts to complications.	Mild	[28]
	9	Higher risk of addiction in male patients.	Mild	[18]
	10	Family/ care givers neglect of the patient maintains addiction.	Mild	[23]
	11	Stigmatization fueled addictive behavior	Mild	[15]
	12	Withdrawal of family and caregivers' support worsen addiction	Mild	[17]
	13	Loneliness aggravate addiction.	Mild	[28]
	14	Enormous physical, psychological, social, and financial burden on the caregivers/ family.	Mild	[23]
	15	Most SCD patients brought up under single parenting care.	Moderate	[26, 28]
	16	Failure to seek specialist attention until complications arise.	Moderate	[17, 23]
	17	Poor academic performance and success associated with addiction.	Severe	[16, 22, 23]
Factors Related to the Society, Religion, and Sociocultural Norms	1	lack of self-help groups and NGO to help in rehabilitation programs.	Mild	[22]
	2	Stigmatization preventing ladies with SCD from getting married.	Mild	[15]
	3	Undue access to drugs due to sociocultural attitudes.	Moderate	[21, 26]
	4	Economic hardship on the family/ caregivers.	Severe	[22, 23, 26]
	5	Antisocial behaviors were exhibited by the addicted patients	Severe	[15, 22, 23]

Discussion

Although Nigeria has a high prevalence (3%) of SCD in newborns, [35] however, that is not a reason to also have a high prevalence of Pentazocine abuse among these patients. The rising prevalence of opioids dependence in SCD patients in Nigeria as observed by clinicians [18,21,22,36] could have been exaggerated. Some of the studies on the prevalence were contradicting, [18] and some results were mere speculations. [37] The high prevalence observed may also be due to pseudo-addiction caused by under-treatment of pain. [17]

The commonest reason why people go to the hospital is "pain" . [41, 42, 43] It was estimated that 90% of hospital admissions for SCA patients was due to painful crises. [44,45] There was a very high correlation between 'disease severity' and 'opiate dependence' in SCA patients. [24] Pain is a multidimensional construct that is influenced by biological, psychological, and social factors. [46,47,48] Patients can individually modulate their perceptions of pain as the sensations ascend to the brain through cultural, emotional, and personal criteria. [49, 50, 51, 52, 53] Equally, every SCA patient has a unique pattern and consistency of crisis, [38,54] and the family Doctor should be able to identify that. However,

most families do not have a family physician, therefore understanding individual patterns could be difficult. Also, it is a routine practice for patients in Nigeria to be changing hospitals or Doctors (Doctor shopping), in search for the best. Some patients use to keep track of certain Doctors and followed them wherever they happen to be.

Higher pain score at triage was found to be associated with admissions and a longer hospital stay in patients with VOC.^[55, 56] Underrating the severity of pain in SCD patients^[23] by the caregivers caused parents to ignore their children complains and increase the chances of development of complications.^[20] While underrating pain by the healthcare team also lead to ignoring patients' genuine complains, reluctance to give adequate analgesia, suboptimal treatment, and ultimately poor management of the patients' condition. Ignoring and underrating patients' pain has caused mistrust between patients and their families/ caregivers/ healthcare team.^[17,41,57] A lot of patients easily learn how to prescribe the drug, forge Doctors' signature, and use multiple prescriptions to acquire large quantity of the drug to be injecting themselves.^[23] Some, especially the ladies, may develop a good rapport with the nearby pharmacy attendants, Doctors, or drug wholesaler, in order to be obtaining a constant supply of the drug easily.^[21] In Nigeria, most of these opioids can easily be bought from chemist/ pharmacy stores without Doctors' prescription.^[16,21,36] In the past, Doctors do not have personal stamps, therefore, their signatures can easily be forged to secure drugs and the government is not doing much in enforcing laws that will address the problems.^[58]

Although management of SCD patients usually involves about 7 goals;^[3] However, most physicians focus on alleviating the pain only. This couldn't be unrelated to the fact that the burden of management of SCD patients relies heavily on general physicians, who may not have adequate expertise to properly manage these patients. Results showed that many Doctors were not used to prescribing narcotic analgesics even in severe pain crises.^[59] Pain control in SCD patients is best achieved with opioids,^[60] and morphine was the drug of choice, followed by acetaminophen, and nonsteroidal anti-inflammatory drugs (NSAIDs). However, morphine was not

commonly available in Nigeria.^[16] Therefore, availability,^[21] affordability, and potency made pentazocine a favorite drug among physicians.^[23,60] Among the drugs prescribed by the Hematologist to SCD patients, 30.1% were analgesics, and 43% of the analgesics were narcotics.^[25] There is also lack of adherence to the WHO/ International Network for the Rational Use of Drugs' (INRUD) guidelines even among the specialist.^[25] Although pentazocine was classified as schedule III drug; however, its abuse potentials could have been underestimated.^[16,61,62] Pentazocine was found to be the commonest abused drug (53.8%) among others (Tramadol, Dihydrocodein, Pethidine, and Morphine), mostly prescribed to patients with SCD in Nigeria.^[18]

The negative image of SCD in the society,^[64] and the long-rooted notion that drug addicts are "bad" people do not only apply to the general populace. Although Doctors are fully aware of the neurobiology of addiction; however, that ingrained notion continue to influence their actions/ inactions toward any patient with a condition associated with chronic pain. Individuals' genetic, environmental, and drug factors may all have roles to play in shaping the development of an individual towards addiction. Genes were found to account for half of a person's risk for addiction.^[65] Gender, ethnicity and mental disorders which are also related to genes may also influence the risks for drug use and addiction. Aside from genetic predisposition, people undergo various forms of stress in their lives, and both conditions were known to precipitate VOC.^[3] Most SCD patients hate their frequent visits to the hospital, except if necessary. In most cases, patients present to the hospital only after self-treatments at home fails.^[3]

Environment plays a vital role in our lives, and it is necessary for our proper physical and mental health developments. The environment of SCD patients has tremendous influences on their health. For example, extremes of temperature, hostile family relations, poor socio-economic status, lack of social amenities, peer pressure, physical and sexual abuse, early exposure to pleasurable drugs, stress, and community norms/ culture and religious backgrounds can greatly affect their health and vulnerability to addiction.^[1] In the past, religion and

culture play vital roles in the lives of people. People used to abide by their traditions, which mostly encouraged proper family ties, good communal relationships, and healthier socio-cultural environment. These days, religion and culture are derogatory; the society ended up with more broken homes, single parenting, and a very harsh social environment. These, together with economic hardships tilt the youth to addiction. Some of the cases of SCD patients reviewed here were brought up by single parents, and few were even raised and continue to live in the orphanage.^[17] Most of the ladies with SCD were single, unmarried, and jobless; even though some had university degrees.^[15] Sickle cell disease patients were often considered as failures, and not everybody wants to associate with them. Marriage could have eased the family's financial burden and also improve the psycho-social wellbeing of the ladies with SCD. However, they were generally stigmatized and rejected by the suitors, employers, and the community in general; simply because they have SCD. So, their lives totally depend on their parents/ caregivers; even though most of them were old enough and capable of starting their own families.

Although Doctors may complain of poor follow-up visits by some patients;^[17] but the truth is, some families cannot afford the specialist consultation fees. Most Nigerians were not covered by the National Health Insurance Scheme (NHIS). As of April 2017, only about 1% of Nigerians were covered by the NHIS, after 12 years of its inauguration.^[66] The patients' neglect by the family, as interpreted by some authors could actually be as a result of the financial hardship imposed by the conditions on the family. Some families avoid frequent hospital visits for either follow up or even during mild crisis due to financial constraints. Some may seek treatment from nearby chemist/ pharmacy in order to cut down cost. Typically, the most effective analgesic for the patient will be used to quickly relieve the patient's pain and the family's agony in any crisis. The family may ignorantly facilitate addiction^[17,19] and give room for more complications to accumulate.^[20]

Both genetic and environmental factors shape the development of an individual. Although taking drugs at any age can lead to addiction; however, the earlier it begins, the more likely it will progress to addiction.

^[67] This is because the areas that control decision-making, judgment, and self-control in the brain (e.g., prefrontal cortex, PFC) is still developing. Exposure to any pleasurable drug will, therefore, cause drug seeking behavior that will be unhindered by the poorly developed PFC. So, it is obvious that a significant number of SCD patients will later become addicts since most of them had multiple exposures to opioids since childhood.

SCD is a condition associated with both physical and psychological stress. The normal physiological and natural stress coping mechanism of the body is highly suppressed in SCD patients. This is as a result of the adrenal insufficiency, a common finding in adults with SCD.^[68] Under normal circumstances, there is diurnal production of cortisol by the adrenal glands in order to maintain normal body functions. In response to stress, the gland produces more cortisol, which prepares the body for "fight or flight response". In adrenal insufficiency however, the gland is not able to produce enough cortisol to meet the body's demands. Adolescence is a period of transition from childhood to adulthood and is associated with enormous stress that requires the release of cortisol and other stress hormones. The combined stress of adolescence and SCD, associated with the adrenal insufficiency may explain why SCD patients are highly prone to addiction at this period of their lives.

Most of the SCD patients reviewed here started taking pentazocine for pleasure during this critical transition period. It is also at this period of transition that most patients lose their support from family/ caregivers. The family/ caregivers' support has since reached its peak and have started to decline gradually with time. It is at this period that adolescent patients start to exhibit the abhorred teenage behavioral problems, making them more distant from the family.^[69] At the same time, there is an increase in social, academic, religious, and SCD demands, etc. All these stressors can easily push a naive SCD teenager to start using Pentazocine to get relief from pain, obtain pleasure, and be more confident among peers.

Many governments including Nigeria, try to combat drug abuse by reducing the drug supply.^[70] A recent crackdown by the Nigerian government to

end drug abuse has led to an increase in the cost of Pentazocine from ≈\$1, 5-10 years ago to about \$2-3 now. ^[71] This was as a result of BBC's documentary on the menace of codeine syrup abuse in northern Nigeria released on 2nd May 2018. ^[71] Pentazocine is now so scarce even for normal uses in hospitals. ^[70]

Conclusion

Nigeria is among the countries with the highest burden of SCD in the world. Addiction to opioids, especially pentazocine was underestimated among these patients. Poor patient/ caregivers' knowledge about SCD and addiction; poverty; mismanagement of painful VOC crisis; low cost, easy accessibility, and availability of Pentazocine; and poor government's regulations were among the most important factors responsible for the addiction. The solution to the problem is a collective responsibility involving the patients, their families/ caregivers, the healthcare team, the society, and most importantly the government. There is an urgent need for action before the menace gets out of hand.

Recommendations

1. Physicians should educate patients and caregivers concerning the disease.
2. Physicians should advise the Government on what is required and also strictly follow the guideline on pain management in SCD patients.
3. Government should enforce laws, provide guidelines, enabling environment, and funding for the successful implementation of health policies.

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Assesment of Some Haematological Parameters in Pregnant Women with Malaria Attending Murtala Muhammad Specialist Hospital, Kano

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Abstract

Malaria is one of today's most devastating diseases affecting most developing countries. Complications associated with malaria infections affect mostly pregnant women and children where it adversely affects the pregnancy outcome. The aim of the study was to assess haematologic parameters of malaria in pregnancy. The case-control study, involving 50 pregnant women with malaria and 30 malaria-free pregnant women, was conducted at Murtala Muhammad Specialist Hospital (MMSH), Kano. Blood samples were collected from the pregnant women and tested for malaria infection and full blood count (FBC). Most women in both the malaria infected and the uninfected groups fell between the ages of 20 to 29 years, and majority of them were Hausa-Fulani. Over seventy percent of the women were multigravida. Education seemed to be the most important socio-demographic characteristics in maternal malaria infection. The results obtained showed that the hematological parameters, especially those determining anaemia were found significantly lowered in malaria in pregnancy. The differences were red blood cell (RBC) ($P=0.006$), mean corpuscular haemoglobin concentration (MCHC), $P= 0.001$, PCV ($P= 0.003$), mean corpuscular haemoglobin (MCH) ($P= 0.25$) and haemoglobin (Hb) ($p= 0.001$). Expectedly, the white blood cell (WBC) count was significantly higher in malaria in pregnancy compared to the control, ($P= 0.001$). In conclusion, these results highlighted the inherent dangers of anaemia in pregnancy as a result of malaria in pregnancy, which portend danger to both the mother and her foetus.

Introduction

Malaria, a mosquito borne infectious disease that affects humans and other animals is caused by species of *Plasmodium* namely: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* as well as *P. knowlesi* [1]. Though classically passed via inoculation by a female anopheles mosquito, malaria parasite can also be transmitted by blood transfusion [2]. In the subtropics and tropical regions of the world, malaria is considered as one of the most important diseases affecting man [3]. The disease accounts for approximately 28.1% of outpatient visits to health facilities, leading to an estimated 13.7% admissions and 9.0% maternal deaths in the endemic regions of the world. Five countries account for nearly 90% of all malaria cases worldwide, with Nigeria accounting for about 25% [4]. Nigeria's annual malaria burden is of 241 million cases of malaria occurring each year in Nigeria [5]. Malaria is particularly disastrous on pregnant women, with about 125 million pregnant women at risk of the

infection each year; in sub-Saharan Africa. Its complications have been reported to have impacts on an estimated 24 million pregnant women worldwide leading to about 500,000 annual deaths of mostly children and pregnant women in the tropics [6,7]. Complications of malaria in pregnancy are among the most devastating negative effects in pregnancy, leading many outcomes such as spontaneous abortion, premature birth, stillbirth, low birth weight, infant mortality and severe maternal anaemia [8 - 10].

Blood and its constituents have vital roles in maintaining life. For example, white blood cells and its differentials are essential in fighting infections, enhancing adaptability to local environmental and diseases prevalent conditions, while Platelets are associated with blood clotting [11]. The total number of red blood cell is determined by the packed cell volume (PCV), which is the percentage of red blood cells in blood. Red cells are involved in the transport

of oxygen and absorbed nutrients, with haemoglobin being central to this physiological function.

Hematological parameters are those parameters that are related to the blood and blood forming organs and are valuable in monitoring the health status of the individual [12,13]. Their importance as indicators of health and disease informs their being studied in this research. Haematological complications are some of the most common complications encountered during malaria infection. These changes involve the major cell types such as red blood cells (RBCs), leucocytes and thrombocytes [14]. Among the major complications of malaria in pregnancy is anaemia. *P. falciparum* malaria infection is said to be the commonest cause of anaemia in Pregnancy [15]. Malaria also leads to changes in both white blood cell count and hemoglobin (Hb) concentrations [16]. Other complications of malaria include significantly lower platelets, as well as alterations in monocyte and neutrophil counts [14,16]. The aim of the study was to assess haematologic parameters of malaria in pregnancy

Materials and Methods

Study subjects

The subjects for the study were 50 pregnant women aged 15 – 40 years, attending Murtala Muhammad specialists' hospital; Kano, Nigeria, who tested positive for malaria. While 30 pregnant women, matched for age, who tested negative were used as controls. Ethical approval was obtained from Kano State Ministry of Health and all participants were required to sign an informed consent form.

Methodology

Interviewer administered semi-structured questionnaires were used to obtain data on socio-demographic variables and reproductive history. Trained medical officers in the hospital assisted in bleeding the women in the morning, following a standard aseptic procedure for blood collection.

Using 5ml syringe the blood sample was taken from median cubical veins and transferred into two EDTA containers to prevent coagulation. One part of the samples were used to test for malaria parasite, and the other part passed into the automatic analyser (Sysmex XN-9000; Hyogo, Japan), for full blood counts, consisting of red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin volume (MCV), Haemoglobin (HB), mean platelet volume (MPV), and red cell distribution widths (RDWs).

Statistical analysis

Mean and standard deviations were used to summarize the data after carrying out normality test. T-test was used for hypothesis testing of socio-demographic variables and chi-square test was used to test the quantitative parameters to compare cases and controls. The data was analyzed using SPSS software version 20. $P < 0.05$ was considered as level of significance.

Results

Table 1 shows the socio – demographic characteristics of the respondents. More 50% of the pregnant women in both the malaria infected and uninfected women were between the ages of 20 to 29 years. In both the case and control groups the respondents were mostly Hausa/Fulani, with only 4% of the cases coming from other Ethnic groups. Most women in the control group had secondary education (46.7%), while those in the case group mostly had no formal education (30%) or had Qur'anic /Adult Literacy (26%). Seventy six per cent of the women infected with malaria were multigravida in comparison with 70% multigravida of uninfected women group. Most respondents in cases (76%) and controls (90%) live in the urban areas.

Table 1: Socio – demographic Characteristics of the Respondents

Characteristics	Controls N= 30 (%)	Cases N=50 (%)
Age (years)		
15 – 19	8 (26.7)	10 (20)
20 – 29	16(53.3)	26 (52)
30 – 40	6 (20)	14 (28)
Ethnicity		
Hausa/Fulani	30(100)	48(96)
Others	0(00)	2(4)
Education		
No formal Education	2 (6.6)	15 (30)
Qur’anic /Adult Literacy	8 (26.7)	13 (26)
Primary	5 (16.7)	10 (20)
Secondary -	14 (46.7)	8 (16)
Post-Secondary	1 (3.3)	4 (8)
Gravidity		
Primigravida	9 (30)	12 (24)
Multigravida	21 (70)	38 (76)
Places of residence		
Urban	27 (90)	38 (76)
Rural	3 (10)	12 (24)

Table 2 shows the distribution of blood cells in malaria infected and uninfected pregnant women. There was lower red blood cells count in malaria infected pregnant women compared to malaria uninfected women. The difference was statistically significant ($\chi^2 = 2.89$, $P = 0.006$). with mean and SD of 3.45 ± 0.79 and 3.97 ± 0.64 for infected and uninfected pregnant women groups respectively. Similarly, the white blood cells counts were also statistically significantly higher in the malaria infected group compared to the uninfected (P -value 0.001, $\chi^2 = -4.702$) with mean $\pm$ SD of 8.18 ± 1.61

for the malaria infected subjects and 10.18 ± 1.41 for the uninfected subjects. Though the mean and SD of the platelet count for the malaria infected pregnant women was higher (175.28 ± 62.17) compared to the mean and SD platelet count of the uninfected group (151.33 ± 51.64), this difference was not statistically significant (P -value = 0.125 $\chi^2 = -1.561$). The results for mean platelet volume (MPV) there was no statistically significant difference between the group ($\chi^2 0.793$ $P=0.432$), with mean $\pm$ SD, 6.58 ± 0.414 for the malaria infected and mean $\pm$ SD 6.65 ± 0.309 for the malaria free group.

Table 2: Distributions of some haematologic parameters in malaria infected and uninfected pregnant women

PARAMETER	uninfected MEAN $\pm$ S.D	infected MEAN $\pm$ S.D	TEST STATISTICS	P VALUE
Red Blood Cell.($\times 10^9$ /L)	3.97 ± 0.64	3.45 ± 0.79	2.89	0.006*
White Blood Cell ($\times 10^9$ /L)	8.18 ± 1.69	10.18 ± 1.41	-4.702	0.001*
Platelets count($\times 10^9$ /L)	151.33 ± 51.64	175.28 ± 62.17	-1.561	0.125
MPV (fL)	6.65 ± 0.309	6.58 ± 0.414	0.793	0.432

* $P < 0.05$

Table 3 shows distributions of hemoglobin concentration (Hb), packed cell volume (PCV), and Red Cell distribution Widths (RDWs). The results of

hemoglobin concentration were found to be significantly lower in malaria infected than in the uninfected participants ($\chi^2 = 2.46$, $P = 0.001$).

Similarly, the results showed that the PCV was reduced in malaria infected pregnant women than malaria uninfected women ($\chi^2 = 2.25$, P-value 0.003.).

In contrast, the RDWs showed no statistically significant difference between the two groups (P-value = 0.130, $\chi^2 = -2.563$).

Table 3: Comparison of Hb, Platelets, and RDWs between infected and uninfected pregnant women.

PARAMETER	uninfected MEAN $\pm$ S.D	infected MEAN $\pm$ S.D	TEST STATISTICS	P VALUE
Hb. Conc. (g/dL)	13.09 $\pm$ 1.37	9.57 $\pm$ 1.29	2.46	0.001*
Packed Cell Volume (%)	34.87 $\pm$ 4.60	29.80 $\pm$ 5.50	2.25	0.003*
RDWs (%)	57.64 $\pm$ 9.68	63.7 $\pm$ 7.45	-2.563	0.130

HB Conc. = Hemoglobin concentration, RDWs = Red cells distribution width

* P < 0.05

Table 4 shows the distribution of red blood cells Indices. The mean corpuscular hemoglobin concentration (MCHC) was statistically higher in malaria uninfected women ($P = 0.001$, $\chi^2 = -3.65$). Similarly, mean corpuscular haemoglobin (MCH) was significantly different ($P = 0.02$) in malaria

infected pregnant women compared to malaria free women. However, Mean corpuscular volume (MCV) levels were comparatively higher in malaria uninfected subjects but the difference was not statistically significant (P-value = 0.062, $\chi^2 = -2.89$).

Table 4.4: Comparison of blood cells indices between infected and uninfected pregnant women with malaria

PARAMETER	uninfected MEAN $\pm$ S.D	infected MEAN $\pm$ S.D	TEST STATISTICS	P VALUE
MCHC (g/dL)	356.7 $\pm$ 4.89	319.4 $\pm$ 24.83	-3.65	0.001*
MCH (pg)	32.55 $\pm$ 7.38	22.98 $\pm$ 3.89	-2.301	0.02*
MCV (fL)	95.84 $\pm$ 7.023	90.5 $\pm$ 6.64	-2.89	0.062

MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, MCV = Mean corpuscular volume.

* P < 0.05

Discussion

The aim of this study was to evaluate some haematological indices in malaria infection of pregnant women compared to malaria-free pregnant women. The results obtained showed that women within the age bracket of 20 to 29 years formed the bulk in both the malaria infected and uninfected pregnant women groups. This is in line with a 2013 study in Northwest Nigeria [17]. Osaro, 2019 [18] found malaria infected pregnant women to be mostly aged 20 – 25 years. The majority of the women attending Murtala Muhammad Hospital were Hausa-Fulani and only 4 percent were of other ethnicities; this could be due to the fact that the hospital is located in a predominantly Hausa-Fulani area and the state itself a predominantly Hausa-Fulani state, with fewer people from other tribes domiciled within the city. Furthermore, most of the participants in this study came from the urban areas of the state. About

90% of the pregnant women in the control group and 76% of those infected came from the urban areas. Majority of the participants were multigravida, in both case and control groups. Education seemed to have a role on malaria infection among the respondents. More than half of the women with malaria had no formal education or had qur'anic /adult illiteracy instructions. On the other hand, majority of those with secondary education (46.7%) did not have malaria in pregnancy. Educational status has a great influence on malaria prevention and control, its lack is a possible risk in malaria infection [19].

Anaemia (defined as Hb concentration of less than 11 g/dL) is a major complication of malaria in pregnancy. This study found significant decreases in all parameters determining anaemia. The study revealed significant decreases in packed cell volume

in malaria-infected pregnant women. Though decreases in packed cell volume during pregnancy are usually due to increase in plasma volume (haemodilution) as a result of the effect of pregnancy hormones, the observed higher levels in this study can be further attributed to the lyses of red blood cells (RBCs). Malaria infection leads to haemolytic anaemia as the plasmodium parasite is known for its entry into and destruction of the RBCs. In line with our finding of decreased RBC, a previous study in 2019 [18] also found decreased RBC count in malaria infection in comparison to that of non-infected pregnant women.

The results of the study showed significant decrease in MCHC, an index of haemoglobin concentration per red cell, with malaria infection. Other studies have found that MCHC levels may change in relation to the levels of malaria parasitaemia; significantly decreasing in patients with moderate parasitaemia and increasing in situations of severe parasitaemia [20]. In a deeper analysis of red cell indices, this study found significantly lower MCH levels in malaria parasitised pregnant women than in malaria-free pregnant women. The mean MCH of the group with malaria in pregnancy was below the standard limit of 26.5 pg - 33.5 pg. These results are in agreement with previous reports. [22]. The observed decrease in MCH could be due to the replication of merozoites which leads to interference with the mechanism of hemoglobin formations in RBCs. In contrast to our findings, another study found elevated level of MCHC in malaria infection, but the elevation was non-significant. [20]

A decrease in the level of MCV was observed in pregnant women infected with malaria, but the observed difference was not statistically significant. In contrast, some previous studies showed decrease in MCV [21]. However, some studies reported higher levels of MCV but showed no evidence relating the increases to pathogenesis of malaria. [16] Furthermore, another study found levels of MCV similar to those found in this study [23].

The results indicated that the platelets counts of both groups studied were within normal values. In fact, the platelets in women with malaria in pregnancy were even higher than the control groups. In contrary to our findings, it has since been shown that the most common complications during malaria

infections are thrombocytopenia (decreased platelet levels) [16]. To buttress this, a number of studies have found that malaria infection leads to significantly lower platelet counts [14]. Though a non-significant negative trend was observed between platelet count and malaria parasitaemia in some studies, severe *falciparum* elicits decrease in platelets count probably due to platelets activation, splenic pooling and decrease platelets life span following malaria infection. The finding of high platelet level in this study could due to bone marrow formation of megakaryocytes to compensate for the low absolute platelet count during acute malaria infection [23, 24].

This study also found significantly higher white blood cells count in the malaria parasitised women group (10.18×10^9 cells/L) compared to the uninfected participants (8.18×10^9 cells/L). The rise in WBC count could be due to increase in immune responses to the parasitic infections. This finding is in agreement with a similar study which found that the WBC of the average adult resident of malaria endemic areas has some immunity to the parasite [25]. Increased levels of WBC may not be found in all cases, as some may present with a mild leucopenia (decreased WBC count), especially in complicated malaria or in adults who are non-immune adults to malaria [26].

Conclusion

Pregnant women infected with malaria exhibited changes in haematological parameters to varying degrees, with significant decreases in all parameters determining anaemia; PCV, MCHC, MCH and a significant increase in WBC counts in the study area. These parameters can be indices for improved malaria diagnosis and effective monitoring of disease and treatment prognosis, in combination with other clinical diagnosis.

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Cardiopulmonary Responses to Exercise in Stroke Patients Attending a Hospital In Jigawa, Northwest Nigeria

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Abstract

Exercise capacity and cardiopulmonary (CP) fitness is seriously reduced after stroke, therefore functional recovery after stroke is a high priority for health care. The aim of the study is to determine the CP parameters in post stroke patients at the spastic and relative recovery stages, before and after 8 weeks of exercise regimens. A total of sixty (60) post stroke patients (34 males and 26 females) matched for age range (30 - 70yrs), sex, and BMI with mean age of 52.80 were randomly selected in to group A and B comprising of subjects in the spastic and relative recovery stages respectively. All patients received 15 minutes aerobic exercise 3 times per week over a period of 8 weeks. Patients were assessed before exercise, and at 4th and 8th weeks of exercise. Stratified random sampling using balloting was employed to select patients. Barthel index scale for assessing functional mobility, modified Asthword scale for grading of spasticity and Bobath stroke recovery staging were used to select both patients in the spastic and relative recovery stages. Cardiopulmonary parameters were compared using paired t-test, taking all P values < 0.05 as significant. There was statistically significant improvement in SBP, DBP, FVC, and FEV1 in all the patients in both groups at 4th and 8th weeks, respectively when compared to 1st week, while FEV1% remain within the range of 74-87%. HR increased slightly at the end of exercise in all the groups. All the groups also showed improvement in HR recovery at 8th week when compared to 1st week. The findings of this study demonstrate that the 8-week exercise regimen improves exercise capacity and cardiopulmonary fitness through a decrease in systolic and diastolic blood pressure, as well as increase in forced vital capacity and forced vital capacity in first second and better heart rate recovery in post-stroke patients in spastic and relative recovery stages.

Keywords: *Cardiopulmonary parameters, Physical exercise, Stroke, Stroke survivors, Stroke rehabilitation*

Introduction

Stroke is condition in which the brain cells suddenly die because of a lack of oxygen.^[1] Stroke is one of the most common causes of long-term disability worldwide, and stroke patients need assistance with activities of daily living, therefore functional recovery after stroke is a high priority for healthcare.^[2] Recovery from stroke is challenging due to impaired neuromuscular control, decreased functional mobility, balance deficits and reduced cardiopulmonary (CP) fitness.^[3] Generally,

respiratory muscle weakness is attributed to the impairment of the muscles involved in respiration, induced by central nervous system (CNS) lesions.^[4, 5] performed a study to evaluate the CP and metabolic responses to maximum exercise and aerobic capacity in 25 hemiplegic patients using upper limb cycle ergometer. The patients showed a significant respiratory dysfunction and reduced exercise tolerance when compared with age matched healthy control group. The result further suggests that a

program of ventilatory and aerobic training should be considered early after stroke to avoid CP problems and deconditioning.

However, aerobic exercise was found to be effective on aerobic capacity (VO_2 peak) regardless of the stage of recovery in post-stroke patients⁶. Depending on the severity of the stroke, the patient may suddenly lose the ability to speak (aphasia) or swallow (dysphasia), there may be memory problem or confusion, visual impairment in one or both eyes, weakness of one side of the face, weakness in an arm or leg or paralysis of the limbs on one side of the body, which can cause difficulty with several daily activities like walking, dressing, eating, etc.^{7, 8} Other complications include incontinence and bladder problem. Cardiovascular disease (CVD) has been a major problem in the developed and developing countries and its burden in these countries is over whelming⁹. Stroke is a common neurological disorder and is the second or third leading cause of death and a major cause of long term disability⁹. Emerging evidence suggests that exercise training in the post stroke population can facilitate improvement in the cardiovascular, respiratory and neuromuscular system¹⁰ Sezer⁵ performed a study to evaluate the CP and metabolic responses to maximum exercise and aerobic capacity in 25 hemiplegic patients using upper limb cycle ergometer. The patients showed a significant respiratory dysfunction and reduced exercise tolerance when compared with age matched healthy control group. The result further suggests that a program of ventilatory and aerobic training should be considered early after stroke to avoid CP problems and deconditioning. However, aerobic exercise was found to be effective on aerobic capacity (VO_2 peak) regardless of the stage of recovery in post-stroke patients⁶.

Emerging evidence suggests that exercise training in the post stroke population can facilitate improvement in the cardiovascular, respiratory and neuromuscular system¹⁰. For a variety of reasons, stroke survivors are not routinely prescribed adequate aerobic and resistance exercises during stroke rehabilitation which likely exacerbates their decline in CP fitness.¹¹ Research studies have demonstrated not only improvement in maximum oxygen consumption (VO_2 peak) after exercise interventions.¹⁰ But also physical function and

psychological well-being or quality of life.^{12, 13}. Yet, many clinicians do not employ exercise interventions appropriately; perhaps because of the limited amount of research that has identified appropriate screening protocols and optimal dosing of aerobic and resistance exercises¹⁴. Cardiopulmonary fitness is important for performing daily activities and mobility. It has been reported that VO_2 peak values of 20 ml/kg/min is associated with limited physical function for instrumental activities of daily living¹⁵. Portempa¹⁶ observed that documented reduction in cardiovascular fitness may contribute to the increase risk of further stroke or myocardial infarction that stroke patients have in comparison with normal subjects. During exercise, there is a progressive increase in the systolic pressure with an increase in VO_2 . Diastolic pressure shows little change with increasing levels of exercise^{17, 18}. If blood pressure falls as exercise intensity increases, a cardiac limitation or abnormality of sympathetic control of blood pressure such as heart failure, ischemia, aortic stenosis, pulmonary vascular disease or central venous obstruction should be suggested¹⁹.

The prevalence of stroke in Nigeria is 1.14 per 1000 population²⁰, and in the middle belt region of Nigeria is 1.31 per 1000 population²¹. The use of exercise in rehabilitation of post-stroke patients in this part of the environment (Birnin kudu, Jigawa State of Nigeria) is common. To the best of our knowledge, there is no report on the cardiopulmonary responses to these exercise regimens in both men and women. Majority of studies reported on the cardiopulmonary response of acute stroke and chronic stroke to separate aerobic exercises, but the combined effect of aerobic exercise and resistance exercise training on the cardiopulmonary function in post stroke survivors at the spastic and relative recovery stage has received little or no attention. In addition, the effect of a prescribed 8-week regimen of the exercise on cardiopulmonary function in post stroke patients has not been evaluated in this environment. The aim of the study was to determine the cardiopulmonary responses in post-stroke subjects at the spastic and relative recovery stages, before and after an 8-week exercise intervention. The result of this research might provide some baseline data on cardiopulmonary responses exercise in post-stroke patients as well add a guide to clinicians in prescribing more effective exercise rehabilitation

protocol for post-stroke patients resident in this environment.

Materials and Methods

Study area

Federal Medical Centre, Birnin-kudu was used as the study area. It is located in Birnin-kudu local Government area of Jigawa state. The hospital is a tertiary institution with multiple speciality units including Physiotherapy unit, which served as the centre for the study. The number of new stroke cases seen in the physiotherapy unit per week ranges from 2 to 5 patients. The number of follow-up cases per day is about 15 to 20 patients. Most of the patients came from near-by towns in Jigawa state and neighbouring Bauchi and Kano states. They predominantly belong to the Hausa and Fulani tribes.

Study population and design

The study population included all stroke patients attending Physiotherapy clinic in Federal Medical Centre, Birnin kudu, who met the eligibility criteria. Every 7th patient was selected to the total of 60 patients into this randomized clinical trial (RCT). Eligible patients were those within the age range of 30 to 70 years, with post-stroke duration of not less than one month and not more than six month, and placed on exercise regimens as part of the routine post-stroke physiotherapy for the first time. In addition, the following inclusion and exclusion criteria were considered: The inclusion criteria; single episode of hypertensive stroke at least 1 month post-stroke; Controlled blood pressure; ability to sit and stand; independent with ambulation can cover > 50 yards (45 m) with or without an assistive device²². patients in the spastic or relative recovery stages. The exclusion criteria; patients with cardiac or other conditions that are absolute contraindications for exercise such as acute cardiac failure, renal failure; patients with history of tuberculosis or any chronic pulmonary condition; patients with unstable blood pressure, cognitive impairment.

Barthel index scale for assessing functional mobility, modified Asthword scale for grading of spasticity, and Bobath stroke recovery staging were used to select both patients in the spastic and relative recovery stages. BMI was determined to select patients with BMI less than 30 kg/m² using the formula: BMI = wt/h² in kg/m².

Procedure for exercise and data collection

The procedure for exercise (squatting) session was also divided into 4 phases, it started with stretching (phase-1) of the quadriceps, hamstring muscles and gluteus to ensure less risk of injury and a better chance of performing the proper technique. Warm-up (Phase 2) began with half squats for a duration of one minute followed by the active phase (phase-3) where subjects were instructed to perform the full squat continuously for 13 minutes with a minimum of 2 to 3 squat per minute, the 4th phase (cool dawn) immediately follows with a half squat which lasted for a minute. The procedure for another form exercise (treadmill) sessions began with phase-1 stretching of the quadriceps, hamstring muscles and gluteus to ensure less risk of injury and a better chance of performing exercise. Subject then stood on the treadmill conveyor belt holding the hand rails, as the motor start rolling, subject took a minutes' walk warm-up (phase-2) on the treadmill at a speed of 0.03 km/h after which the exercise intensity was increased gradually to the prescribed speed of 0.1 km/h (minimum speed required to maintain 50-85% of maximum heart rate) for 13 minutes (phase-3/active phase), then a minute walk cool-down (phase-4) was employed before terminating the exercise.

Participants completed 8-weeks exercise intervention for 15 minutes duration 3 times per week. Cardiopulmonary (CP) response parameters were measured before the commencement of exercise in the first week, then after exercise at the fourth week and eight week. Post exercise vital signs were measured to ensure a return to pre-exercise values. Measurements were taken at rest before the commencement of exercise, at the peak of the exercise and 5 and 20 minute after the exercise.

The cardiopulmonary parameters included systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, (HR), forced vital capacity (FVC), and forced expiratory volume in one second (FEV1).

Automated digital arm BP monitor was used to measure the heart rate, with the patient seated, relaxed and not speaking, the monitor which was already neatly placed on the exposed left arm and supported at the level of the heart was turned on by selecting the appropriate button, which automatically allows the cuff to inflate and deflate. The monitor could detect the heart rate as the cuff deflates and

displays the measurement on a screen. Sphygmomanometer was used to measure the blood pressure (SBP and DBP), immediately after taking the HR, arm cuff connected to the sphygmomanometer was neatly placed round the left arm about 2.5-5 cm above the cubital fossa with the centre of the bladder over the brachial artery. Stethoscope bell was placed over the brachial artery below the antecubital space before inflating the cuff. The cuff pressure was gradually released by slowly opening the air release knob. The first sound was recorded as the SBP and DBP was recorded when the final muffled sound disappear.

Spirometer "DATOSPIR 120" was used by the researcher to measure the FVC, and FEV1 in accordance with the recommendations of the Datospir user manual. Patients' demographic and anthropometric characteristic was assessed before applying ventilatory function test. Subjects seated before the measurement, after appropriate placement of nose clip and mouth piece each subject was asked to do a forced quick expiration after maximum inhalation and end the manoeuvre with inhalation. After doing at least three acceptable and repeatable FVC manoeuvres the largest FVC and FEV1 were recorded.²⁴ and ²⁵ and calculate the FEV1/FVC ratio.

All measurements were taken with the help of well-trained assistants.

Ethical consideration

The study was conducted in accordance with the institutional guidelines after the approval from the Health Research Ethics Committee, Federal Medical Centre, Birnin Kudu, Jigawa State, (Ref. No. HREC/0001/2015, dated 23/06/2015). An informed written consent was obtained from each subject after they were informed about risk and benefits of participating in the research.

Statistical analysis

Data were collated and analysed using the SPSS version 20.0 statistical software (IMB Inc., Chicago, and U.S.A). ANOVA was used to compare mean values taken before the commencement of the exercise and at the 4th and 8th weeks. Values are expressed as mean $\pm$ S.E.M. P values < 0.05 were considered significant.

Results

Socio-demographic Characteristics of Participants

A total of sixty (60) patients with stroke (34 Males (56.7%) and 26 Female (43.3%)) participated in the study as shown in figure 1

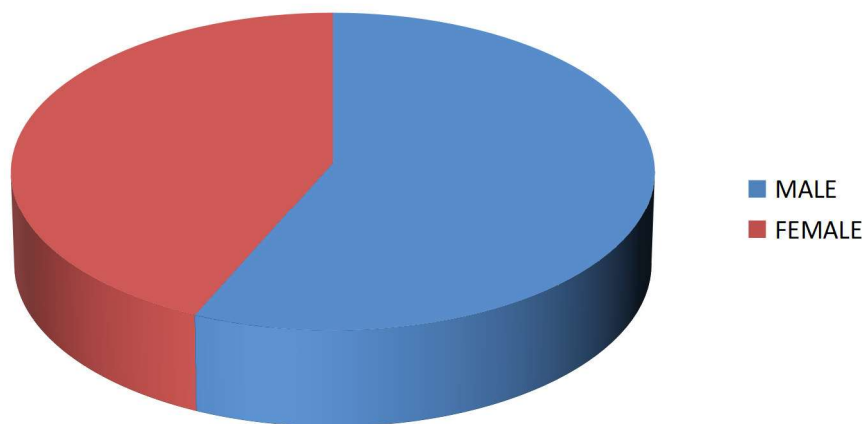


Figure 1: *Distribution of the research participants by sex*

The mean age of the participants was 52.80 years, males had 55.62 years and females had 49.12 years, those in the spastic stage and relative recovery stage

had mean age of 52.63 and 52.97 years, respectively. Figure 2 shows the mean age of subjects.

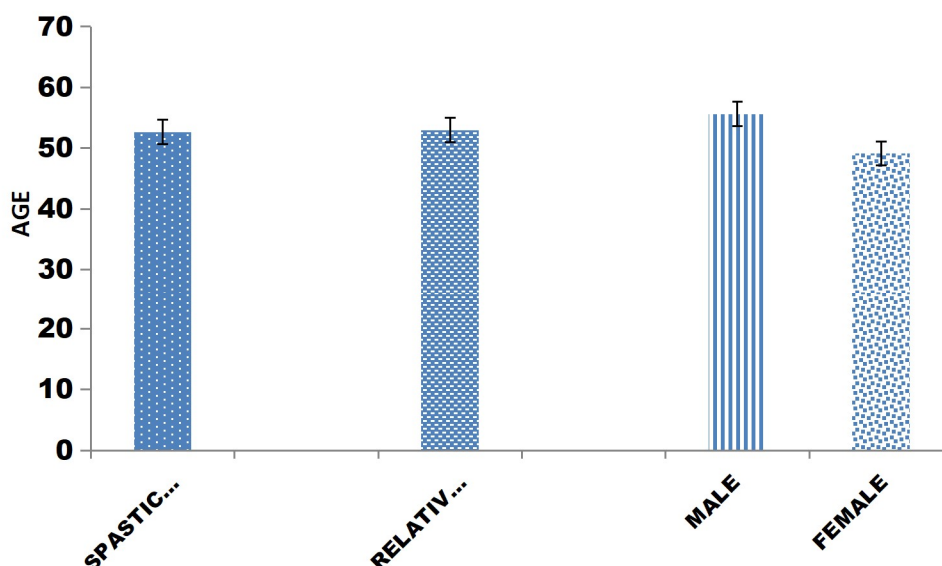


Figure 2: Distribution of sex and stage of stroke recovery of the participants by age

85% of the subjects were from the low income socioeconomic status only 15% were in the middle

class and none were from the high income class as seen in figure 3.

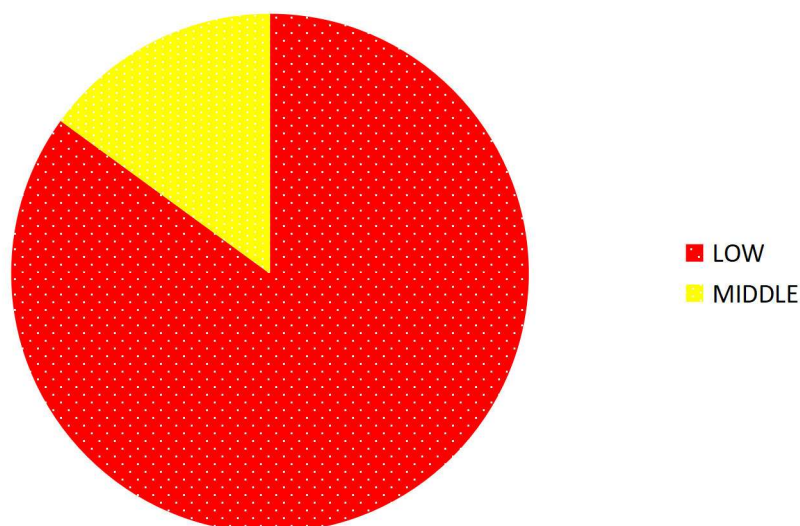


Figure 3: Distribution of socio-economic status of the participants.

Anthropometric characteristics

The mean BMI of participants was 22.25kg/m² (male 22.42 and female 22.03), males are a little bit taller also having more weight (1.73m² – 65.74kg) than the females (1.67m² – 61.12kg). Those in the

spastic stage had BMI of 22.68kg/m² as compared with 21.82 for participants in the relative recovery stage. Figure 4 shows the participants BMI across stroke recovery stages and gender.

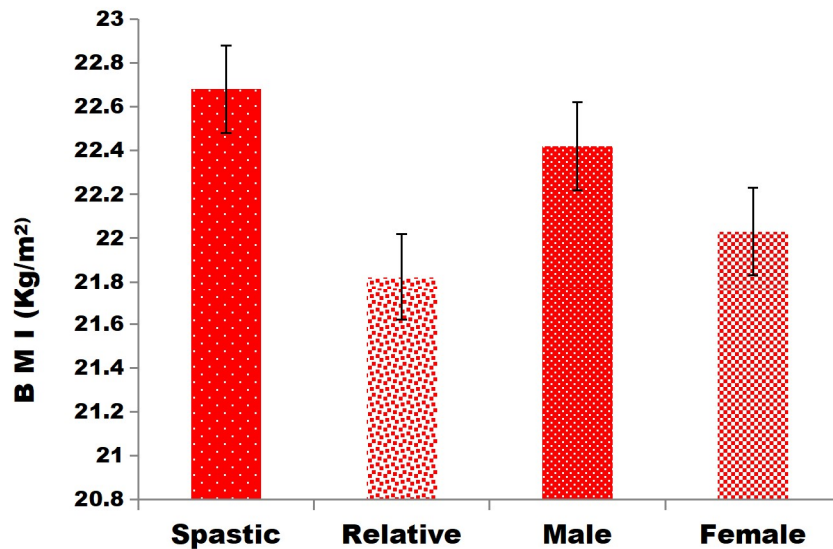


Figure 4: Distribution of body mass index (BMI) of the participants according to sex and stage of stroke recovery.

Stroke characteristics

Table 1 presents the participant's stroke characteristic. Barthel index score for functional mobility was used to assess subjects pre and post

exercise, significance difference ($p < 0.05$) was seen among the two groups at 8-week in figure 5

Table 1: Stroke Characteristics of the Study Participants ($n=60$)

Variables	Number	Percentage
Type of stroke:		
Heamorrhagic	43	71.70
Ischeamic	17	28.30
Side weakness:		
Right	46	76.70
Left	14	23.30
Stroke Recovery stage:		
Spastic Stage	30	50.0
Relative Recovery stage	30	50.0
Medication:		
Bêta-blockers	11	18.30
ACE inhibitors	12	20.0
Calcium channel blockers	11	18.30
Combined medications	23	38.30

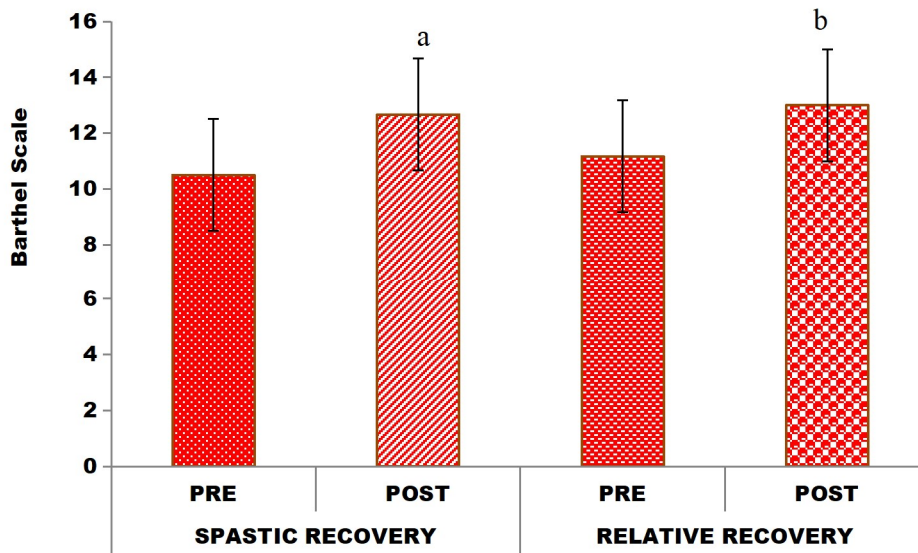


Figure 5: Barthel Scale for functional mobility among the study participants.

a = significant ($p < 0.05$) between (Pre exercise) and week 8 in the spastic group, b = Significant ($p < 0.05$)

between (Pre exercise) and week 8 in the relative recovery group.

Effects of exercise on cardiovascular response of the Post-Stroke patients who participated in the study

As shown in Figure 6, there was statistically significant difference ($p < 0.05$) in the SBP, DBP, HR, at week 4 and 8 when compared to week 1.

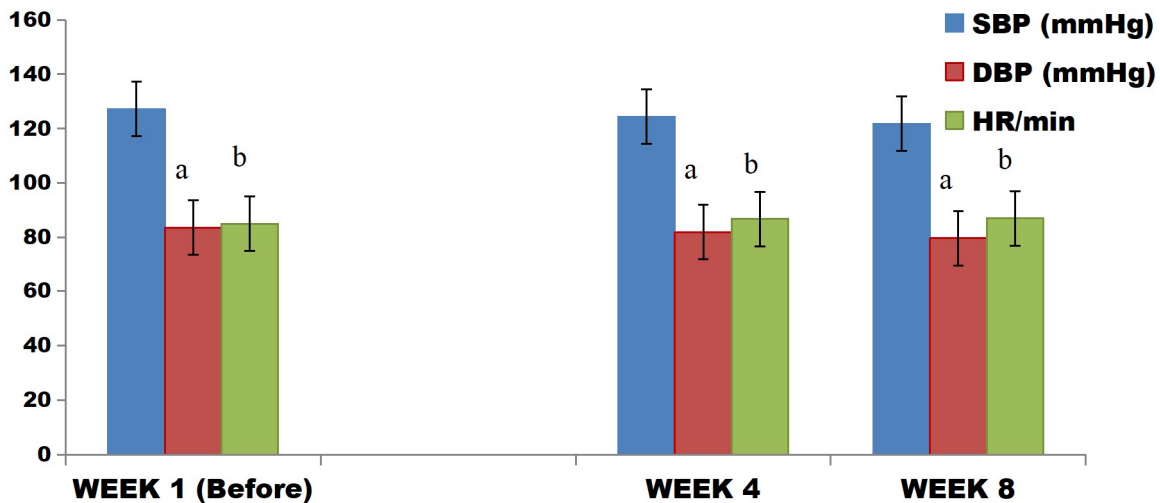


Figure 6: Effect of exercise on cardiovascular parameters of all the post-stroke patients after an 8-week exercise protocol (Mean $\pm$ S.E.M.) ($n=60$)

a = significant ($p < 0.05$) week 4 compared to week 1 (before exercise), b = significant ($p < 0.05$) week 8 compared to week 1 (before exercise).

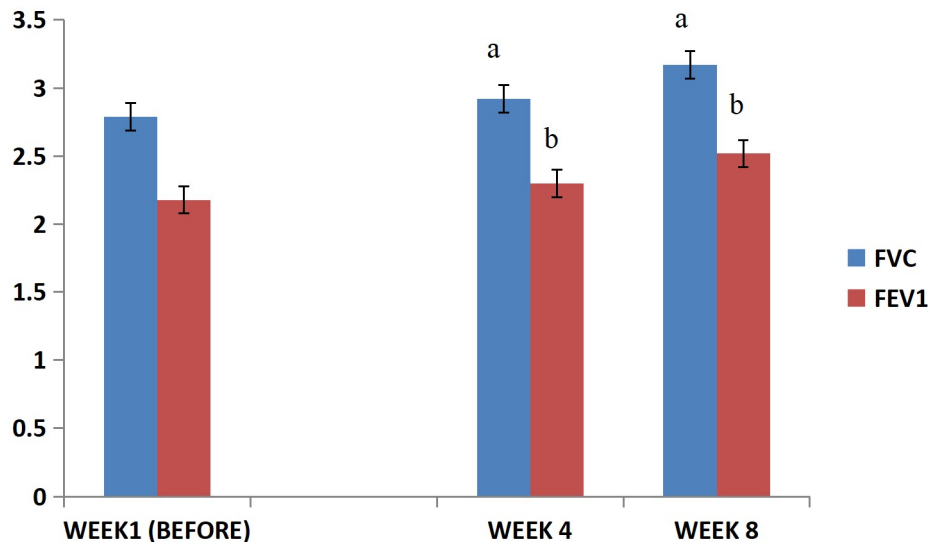


Figure 7: Effect of exercise on lung function parameters of all the post-stroke patients after an 8-weeks exercise protocol (Mean $\pm$ S.E.M.) (n=60)

a = significant ($p < 0.05$) week 4 compared to week 1 (before exercise), b = significant ($p < 0.05$) week 8 compared to week 1 (before exercise).

Discussion

Emerging evidence suggests that exercise training in the post stroke population can facilitate improvement in the cardiovascular, respiratory and neuromuscular system¹⁰. This study aimed to determine the cardiopulmonary responses parameters in post stroke patients at the spastic and relative recovery stages before exercise and after 8 weeks of exercise regiments were done

All the patients for this research have successfully completed the exercise protocol except 2 patients who interrupted their exercise for 2 sessions due to elevated blood pressure and return after the blood pressure was stable. The patients in the two groups and sub-groups showed significant decrease in pre-exercise SBP (by 3 mmHg) and DBP (by 2 mmHg), increase in FVC (by 0.13L) and FEV1 (by 0.12L) at week 4 when compared to week 1. These changes are normal-positive physiological responses to prolong exercise showing that physical exercise protocol has been effective on the cardiac and respiratory muscle performance enhancement of the post-stroke patients in all the groups, leading to an

improvement in the cardiovascular conditioning. Physical exercise delivers mechanical energy to the human body. The subject actively walk/run this process gives the person effective cardiovascular and pulmonary work out through the involvement of all the antigravity muscles.^{26, 27} WilBorn²⁸ conducted study on 16 patients undergoing alternate inverted leg press and upright hack squat using 1RM (65% to 85% of 1RM) to grade the exercise intensity in both groups, the findings suggest increase in HR, SBP, and DBP, and a return to the resting value after exercise. The cardiovascular measures are influenced by intensity of the exercise, rather than apparatus or change in body position during resistance exercise. The work by WilBorn was in agreement with our present study.

Kim^[23] reported the effect of individualized respiratory muscle training device combined with conventional physical therapy exercise on the pulmonary function and exercise capacity of 20 stroke patients (10 experimental; 10 controls). Improvement and a significant intergroup difference were observed in the FVC, FEV1 and peak expiratory flow after exercise. His findings suggest that exercise of the respiratory muscle using an individualized respiratory device combined with other forms of conventional exercise had a positive effect on pulmonary function and exercise capacity than the conventional physical therapy exercise alone. This findings support study reported by Forjaz²⁹ on 16 normotensives performing ergometry

exercise at varying intensities to determine the influence of exercise on the blood pressure (BP) and HR. They found out that exercising at lower or higher intensities lead to decrease in BP after exercise and at lower intensity the HR decreases but at higher intensity the HR increases after exercise.

Conclusion

The findings of this study demonstrate that the 8-week exercise regimen improves exercise capacity and cardiopulmonary fitness through a decrease in systolic and diastolic blood pressure, and increase in forced vital capacity and forced vital capacity in first second, as well as better heart rate recovery in post-stroke patients in spastic and relative recovery stages. Percentage forced vital capacity in the first second was within normal range.

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Comparative studies of Effects of Stem Bark Extracts of *Moringa oleifera* and *Ficus platyphylla* on Hematologic and Oxidative Stress indices of Phenylhydrazine-induced Anemic Rats

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Abstract

This study was aimed to investigate and compare the possible effects of aqueous stem bark extracts of *Moringa oleifera* and *Ficus platyphylla* on blood parameters and some indices of oxidative stress; such as reduced glutathione and Malonaldehyde concentrations. Thirty-five (35) Wistar rats of both sex were randomly divided into 5 groups: Group 1 were untreated controls; Group 2 received only Phenylhydrazine: negative controls; Group 3 received Phenylhydrazine +200 mg/kg of *Moringa oleifera* extract; Group 4 received Phenylhydrazine +200 mg/kg of *Ficus platyphylla* extract; Group 5 received phenylhydrazine + 200mg/kg of the extracts combined. The results of this work indicate a significant decrease in PCV (%), HB (g%), RBC, MCV and C.I for those rats that received only Phenylhydrazine (Group 2) when compared to control group with 30.60 ± 0.87 , 8.88 ± 0.36 , 2.77 ± 0.23 , 112.47 ± 8.78 , 1.12 ± 0.09 Vs 39.80 ± 0.66 , 19.00 ± 0.32 , 5.60 ± 0.39 , 72.62 ± 5.69 , 0.73 ± 0.06 , respectively. However, administration of the extracts significantly increased the mean values of all red blood cell parameters, and lowered the Malondialdehyde concentration amongst all the Groups 3,4 and 5 rats respectively as compared to those in group 2. Therefore, aqueous bark extract of *Ficus platyphylla* was observed to be more effective than *Moringa oleifera*, and the combination of these extracts was however observed to be highly effective than the individual extracts.

Keywords: Hemolytic Anemia, Stem Bark Extract, *Moringa oleifera*, *Ficus platyphylla*, Erythrocytes, Malondialdehyde, Glutathione

Introduction

Anemia can be defined as an abnormal condition of the body in which hemoglobin (Hb) concentration and/or red blood cell (RBC) counts (and subsequently their oxygen carrying capacities) are lower than normal and insufficient to meet the physiological needs of the body [1]. It is the commonest red cell disorder that occurs mainly secondary to other conditions such as malnutrition, genetic or acquired defects, parasitic infections, blood loss, as well as drug toxicity, affecting different age categories of people with elderly, young females and infants being more at risk [2]. Anemia is usually characterized by decrease in hemoglobin concentration below normal values for a person's age, gender, and environment, and at times accompanied by a decrease in the red cell count below normal values [3]. Considering the fact that it is a common blood disorder affecting all the age groups, anemia is a major concern in a global

healthcare. In African countries, the main causes of anemia are due to poor nutrition and malaria [4]. Beside malaria, exposure to drugs (e.g Haloperidol), toxins (e.g snake venoms) and chemicals (e.g phenylhydrazine) can also lead to hemolytic anemia. For example, phenylhydrazine causes hemolytic anemia by oxidative damage to erythrocytes leading to decreasing hemoglobin level, red blood cell concentration, and packed cell volume [5].

In different parts of the world, medicinal plants have remained a very significant alternative source of drugs for majority of people with poor access to orthodox healthcare facilities [6]. *Moringa oleifera* (*M. Oleifera*) also called "zogale" in Hausa language is the most widely cultivated species of a monogeneric family, the moringaceae that is very common in the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan, which has

been used for traditional medicine and industrial uses [7]. *Moringa oleifera* is rich in simple sugar, rhamnose, glucosinolates and isothiocyanates [8]. It has also been reported that all parts of *Moringa oleifera* are used in the treatment of diseases such as diabetes, scurvy, intestinal worms, diarrhoea, headache, earache, toothache, skin rashes and abrasion, wounds, ulcers, bronchitis, and anemia [9].

Similarly, *Ficus platyphylla* (Moraceae) is a deciduous plant called “gamji” in Hausa, it is widely distributed in the savannah region of West African coast. Preliminary toxicity and phytochemical studies of the stem bark extracts shows that *Ficus platyphylla* has potential hemopoietic effects [10,11]. Preliminary phytochemical screening revealed the presence of flavonoids, tannins and saponins [12].

Phenylhydrazine (PHZ) is a chemical compound used worldwide as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. PHZ and its derivatives were used earlier as antipyretics but their hematotoxic action made their use dangerous [13].

Various diseases including anemia were successfully treated using different medicinal plants by traditional herbalists with no proper scientific evidence [11]. Hence, a proper scientific and laboratory evidence of these medicinal plants on their pharmacological and potential toxicological properties to confirm their clinical effects and safety is indeed needed. Therefore, this work aimed at studying the anti-oxidant and anti-anemic effects of aqueous stem bark extracts of *Moringa oleifera* and *Ficus platyphylla* on phenylhydrazine-induced anemic Wistar rats

Materials and Methods

Sample Collection and Preparation

The stem bark of *Moringa oleifera* and *Ficus platyphylla* were obtained from the rural region of Rogo local government area, Kano state, Nigeria. They were named and authenticated by a plant taxonomist in the department of Plant Science, Bayero University, Kano. Herbarium accession numbers were assigned as BUKHAN 11 and BUKHAN 106 for *Moringa oleifera* and *Ficus platyphylla* respectively.

Phenylhydrazine (PHZ) was obtained from loba chemie pvt., Mumbai, India. All other chemicals and

reagents were of analytical grade and where obtained from Human Physiology Department, Bayero University, Kano.

Phytochemical Analysis

The Bioactive components of the plants were extracted based on the procedure described by Handa [14].

The powder of the stem bark (200g) was added separately in to 1000mL conical flask followed by addition of 500mL distilled water and the mixture was left at room temperature for 3 days [14]. Extraction was carried out without adding any heat to avoid compound destruction [15]. Both extracts were evaporated under vacuum at 40°C using vacuum rotary evaporator to obtain concentrated extracts. The extracts were transferred into air tight amber bottles and stored at 20°C.

Phytochemical result showed that stem bark extract of *Moringa oleifera* and *Ficus platyphylla* possestannins, saponins, carbohydrates, glycosides, triterpenses, flavonoids and steroids.

Experimental Animals

A total of 35 adult (male and female) rats aged 4-6 weeks and weighing 130-170g obtained from Anatomy Department, Bayero University Kano were used in this work.

All animals in this study were handled in accordance with the ethical standard of the Organization of Economic co-operation and development (OECD) Guidelines for Animals Experimentation (OECD No.425) [16].

Determination of Lethal Dose (LD50):

Acute oral toxicity study was done on healthy Wistar rats according to OECD guideline no. 425 [16]. The LD50 of *Moringa oleifera* and *Ficus platyphylla* were found to be greater than 2000 mg/kg bw and 3000 mg/kg b.wt, respectively,

Animal Grouping

The rats were divided into five groups consisting of five animals each, group 1 serves as control, group 2 received only 40 mg/kg body weight of Phenylhydrazine and serves as negative control, group 3 received phenylhydrazine and 200 mg/kg body weight of aqueous stem bark extract of *Moringa oleifera*, group 4 received phenylhydrazine and 200mg/kg body weight of aqueous stem bark extract of *Ficus platyphylla* and group 5 received phenylhydrazine and 100 mg/kg body weight of

Moringa oleifera + 100mg/kg body weight of *Ficus platyphylla* extract for 14 days.

Induction of Anemia, Administration of Plant Extract and Collection of Blood samples

A modified method described by Berger [17] was used in this study. A dose of 40 mg/Kg of PHZ-HCl was administered by intraperitoneal injection for two days. On the 3rd day, their blood samples were collected by a little prick at the tail veins of each rat in to heparinized capillary tubes for hematological analysis. Rats with packed cell volume of < 30% were considered anemic and were used in this study [18]. After anemia induction, the experimental groups were subjected to overnight fasting, and on 4th day 200mg/Kg body weight of aqueous stem bark extracts of *Moringa oleifera*, *Ficus platyphylla* and combined extracts were administered to rats in group 3, 4 and group 5 respectively for 14 days. Group 2 was the negative control group, the rats were anemic just like groups 3, 4 and 5 but they were not treated with either of the extracts, while group 1 were normal healthy rats. On day 15, the rats were placed under chloroform anesthesia after which they were sacrificed and blood samples were collected via direct cardiac puncture. About 2mL portion of the blood sample was immediately transferred into EDTA bottles for hematological analyses, the remaining portion of about 3mL was placed into plain sample bottles, allowed to clot and the serum was carefully obtained for determination of reduced glutathione (GSH) and malondialdehyde (MDA) concentrations.

Determination of Hematological and Oxidative stress Parameters

Estimation the of red and white blood cells, hemoglobin concentration, packed cell volume and differential white blood cells were determined using routine laboratory methods described by Lewis and Decei [32].

Reduced glutathione (GSH) was determined by the method of Ellman [19]. 0.5 ml serum precipitated by 2 ml of 5% TCA and 1 ml of supernatant was withdrawn and 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer was added to the supernatant. Following a yellow color development, absorbance was taken at a wave length of 412 nm. Malondialdehyde (MDA) was measured by the method of Albrow and Das [20, 21]. About 0.1 ml of serum was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was transferred in to fresh plain tubes. Following a pink yellow color development, absorbance was then taken at a wavelength of 535nm.

Statistical analysis

The results obtained in this work were all express as Mean $\pm$ SEM and the statistical variations were evaluated using one way Anova at p value of less than 0.05 and Bonferroni post hoc test respectively.

Results

Hematological indices

The result for the hematological test reveals differences in mean values of blood cell parameters between the control and experimental groups as indicated on table 1. The red blood parameters for the groups that received the extracts were found significantly increased signifying that the extracts possess anti anemic activities.

Table 1. Comparison of red blood cell parameters between the control and experimental groups

Parameters	Control	Untreated anemic	<i>Moringa o.</i>	<i>Ficus p.</i>	<i>Moringa + Ficus</i>
PCV(%)	39.80 $\pm$ 0.66	30.60 $\pm$ 0.87*	39.50 $\pm$ 1.20 ^a	41.60 $\pm$ 1.72 ^a	45.00 $\pm$ 1.58 ^a
HB(g%)	19.00 $\pm$ 0.32	8.88 $\pm$ 0.36*	15.50 $\pm$ 0.95 ^a	16.88 $\pm$ 1.29 ^a	19.20 $\pm$.49 ^a
RBC	5.60 $\pm$ 0.39	2.77 $\pm$ 0.23*	5.39 $\pm$ 0.27 ^a	5.60 $\pm$ 0.27 ^a	5.84 $\pm$ 0.28 ^a
MCV	72.62 $\pm$ 5.69	112.47 $\pm$ 8.78*	73.94 $\pm$ 3.02 ^a	75.00 $\pm$ 4.82 ^a	77.88 $\pm$ 1.54 ^a
MCH	34.66 $\pm$ 2.68	32.52 $\pm$ 2.173	28.59 $\pm$ 0.95	30.55 $\pm$ 3.02	33.06 $\pm$ 1.18
MCHC	47.74 $\pm$ 0.25	29.15 $\pm$ 1.68*	39.00 $\pm$ 2.06 ^a	40.42 $\pm$ 1.97 ^a	42.77 $\pm$ 1.00 ^a
C.I (%)	0.73 $\pm$ 0.06	1.12 $\pm$ 0.09*	0.74 $\pm$ 0.03 ^a	0.75 $\pm$ 0.05 ^a	0.81 $\pm$ 0.03 ^a

*P<0.05 Vs normal control group and ^aP<0.05 Vs Untreated Anemic Values expressed as $\pm$ SEM

Table 2. Comparison of total and differential white blood cell counts among the groups

Parameters	Control	Untreated anemic	<i>Moringa o.</i>	<i>Ficus p.</i>	<i>Moringa + Ficus</i>
WBC 10 ⁹ /L	7.92±0.76	11.24±0.27	11.61±0.76*	10.60±0.5	9.45±1.21
NEUT (%)	38.00±1.14	79.80±2.92*	68.00±1.65	63.20±2.27	56.20±3.48
LYMP (%)	58.60±0.75	17.40±2.96*	26.17±1.97	33.60±1.80 ^a	39.60±2.56 ^a
MONO (%)	3.40±0.51	2.60±0.68	5.83±1.17	3.20±0.58	4.20±0.97

*P<0.05 Vs normal control group and ^aP<0.05 Vs negative control group. Values expressed as ± SEM

The result also shows mean differences in total and differential white blood cells count of both control and experimental groups as indicated on Table 2, and there was significant increase in neutrophils and decrease in lymphocytes counts in untreated anemic rats compared to normal control group. There was

however significant increase in lymphocytes count in *Moringa oleifera* (*Moringa o.*) and *Ficus platyphylla* (*Ficus p.*) groups compared to untreated anemic rats of group 2 (P=0.018). And no significant difference in Monocyte count was found between the entire groups (P>0.05).

Table 3: Comparison of serum MDA and GSH (expressed in μ mol/L) among the groups

Parameters	Control	Untreated anemic	<i>Moringa o.</i>	<i>Ficus p.</i>	<i>Moringa + Ficus</i>
MDA	153.80±15.79	139.40±13.56	99.20±9.84	95.00±17.34	70.60±8.24 ^a
GSH	12.74±1.33	12.50±1.54	13.02±1.30	14.59±0.96	13.09±1.68

*P<0.05 Vs normal control group and ^aP<0.05 Vs negative control group. Values expressed as ± SEM

The oxidative stress parameters for the anemic rats were evaluated through the determination of malondialdehyde and reduced GSH. The result shows significant decrease in MDA concentration in group of rats treated with combined extracts when compared to control group and untreated anemic group (P=.002), there is also insignificant decrease of MDA in *Moringa oleifera* and *Ficus platyphylla* groups in comparison to control group and untreated anemic group. Also, mean values of GSH in group 3, 4 and 5 were higher than that of group 1 and 2, the difference was however not statistically significant as indicated on table 3.

Discussion

In this study, the hemopoetic and antioxidative effects of aqueous stem bark extracts of *Moringa oleifera* and *Ficus platyphylla* on phenylhydrazine induced anemic rats were studied.

Acute Toxicity Study

The acute toxicity study of these plants was carried out as described by OECD [22]. The result revealed that, the aqueous extract of *Moringa oleifera* and *Ficus platyphylla* showed no evidence of toxicity even at higher doses after administration of the extracts. Only mild sedation and salivation were observed in *Moringa oleifera*-treated group, while

mild sedation and minor hair loses were observed in *Ficus platyphylla* treated group. Therefore, the observed high LD50 of *Moringa oleifera* and *Ficus platyphylla* indicates that the barks of these plants are safe for animal consumption [27,33].

Phenylhydrazine (PHZ) and its derivatives such as 2,4-DNPH induced hemolytic anemia is a validated methods used to determine the anti-hemolytic properties of drugs in experimental animals [23]. Phenylhydrazine (PHZ) induces hemolysis of red blood cells (RBCs) by inducing the formation of toxic free radicals that can attack cellular macromolecules such as hemoglobin, resulting in the oxidative damage within the RBCs and oxidative degradation of spectrin in the membrane skeleton resulting in their destruction.

According to the findings of our study PHZ significantly decreases Hb level, RBC concentration, PCV, MCH and increases the mean MCV. As supported by different literatures this is a clear indication of hemolytic action of PHZ on red blood cells, the anemia induced is macrocytic hypochromic in nature [1, 23,24].

Hematological assessment

Administration of 200mg/kg body weight of aqueous stem bark extract of *Moringa oleifera* for 14 days

reversed the anemic condition of these rats by significantly increasing the values of PCV, Hb, and RBC when compared to the anemic untreated group. Our findings are not consistent with that reported by Reddy *et al.* [27] that stem bark extract of *Moringa oleifera* shows no significant effect on RBC and WBC counts, the inconsistency may be as a result of the different phytochemicals with what is reported in our study, additionally, the methodology and models used were different.

The result shows significant elevation of PCV, RBC, and Hb in group treated with 200mg of *Ficus platyphylla* for 14 days when compared to anemic untreated group. This result is in agreement with findings of Ndatsu [11] that reported significant elevation of all hematological indices such as RBC, Hb and PCV in a group treated with stem bark extract of *Ficus platyphylla* when compared to untreated rats. Our findings are further supported by the work of Isma'il [28] who reported that mice in the infected, untreated group showed drastic reduction in the mean values of PCV as the infection progresses, this was however prevented significantly ($P < 0.05$) in mice treated with the ethanolic stem bark extract in comparison with PCV values obtained from the infected, untreated group.

Main reasons for the potential ameliorative potential of the aqueous extracts of *Moringa oleifera* and *Ficus platyphylla* are likely to be due to the chemical constituents of the plants. Alkaloids and steroids for instance, are known to inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase enzyme thereby causing an accumulation of cAMP levels; this stimulates protein phosphorylation and synthesis with a possible enhancement of erythropoiesis Ndem *et al.*, [30]. Saponins and triterpenses were also found to play role in a cell signaling processes Gauthier *et al.* [32].

This study shows non-significant increase in WBC count in *Moringa Oleifera* treated group when compared to the negative control group, but was found to be higher than in the group of rats treated with *Ficus platyphylla* and the extracts combined. Our finding is in line with that reported by Reddy *et al.* [27] and Ufelle *et al.* [25], that no significant change in total white blood cells count after weeks of *Moringa oleifera* administration. The result is also in agreement with that reported by Ari-egoro *et al.* [26]

that also reported an increase in lymphocytes and monocyte with decrease in neutrophils count after two weeks of treatment with *Moringa* compared to negative control group. Our findings of increased in lymphocytes and monocytes with decrease in neutrophils are not consistent that of Ufelle *et al.* [25]. This difference may be attributing to variation of phytochemical constituents of stem bark and leaves. Increase in lymphocytes in groups treated with *Moringa oleifera* extract may indicate the function of differential white blood cell (Lymphocyte) to fight against invading foreign body as was phenylhydrazine Ari-egoro *et al.* [26].

Even though the effect is not statistically significant, administration of *Ficus Platyphylla* for two weeks after Phenylhydrazine injection shows a tendency to reverse the effect by decreasing the WBC count, neutrophils and increasing the lymphocytes counts compared to the anemic untreated rats. Our finding is not in agreement to the finding of Chinenye *et al.* [10] that reported decrease in lymphocytes. Effect of *Ficus platyphylla* extract on decreasing the total WBC count and increasing lymphocytes may be attributed to its immune modulatory effects, and indicates its potential actions to fight infections.

Oxidative stress Indices

As observed from untreated anemic rats, our study confirms hematotoxic effects of PHZ administration on red blood cells. However, no statistically significant difference in serum concentrations of reduced glutathione and malondialdehyde were found between untreated and normal control group. This result is not in line with the findings of Ashour [24] and Kolawole *et al.* [29] that rats treated with PHZ shows an increase in MDA concentration and decrease in GSH levels compared to normal rats. This change in MDA and GSH may occur due to the natural antioxidant of the body of the rats to lower their MDA level within the 14 days of the study period.

Also the result is supported by the fact that phenylhydrazine induced hemolytic anemia in animals by oxidative degradation of erythrocytes proteins not by lipid peroxidation Berger [5]. The administration of the aqueous stem bark extract of *Moringa oleifera* and *Ficus platyphylla* have resulted in increasing the concentration of reduced GSH with a concomitant decrease in

malonaldehyde levels across the groups. The ability of these extracts to decrease MDA and increase GSH concentration indicates their anti-oxidant potency. Comparably it can be observed that group of rats treated with *Ficus platyphylla* showed a higher tendency of reducing the mean values of MDA and increasing the levels of GSH when compared to *Moringa oleifera* treated rats. The result of this work clearly indicate that the aqueous stem bark extract of *Ficus platyphylla* possess more anti oxidant activity than that of *Moringa oleifera*, even though the combination of the extracts seems to be more effective.

The results of the present study clearly suggest possible potential benefit of the stem bark aqueous extracts of *Moringa oleifera* and *Ficus platyphylla* and especially when combined together on some indices of enzymatic antioxidant status (reduced glutathione), lipid peroxidation and oxidative stress (malondialdehyde) following the administration of PHZ in our experimental animals. It has been reported that flavonoids, present in the aqueous bark extracts of these plants possess the ability to protect cells as antioxidants and thus repair damage to red blood cells by preventing generation of free radicals or highly reactive oxygen species Asgary [31]. Further studies are needed to characterize and explore the biological activities that may be present in these extracts to formulate an effective herbal preparation that will be used to combat drug induced anemia in animal models.

Conclusion

The hemopoetic and antioxidant effect of aqueous stem bark extract of *Moringa oleifera* and *Ficus platyphylla* were studied. The result revealed that none of the extract is toxic but were found effective in improving hemopoiesis and have good antioxidant properties. By comparison, *Ficus platyphylla* was found to be more effective in improving the red blood cell indices, the rats' body immune functions and antioxidant potential when compared to *Moringa oleifera*. However, combination of these extracts was found to be more effective, showing a possible synergistic effect.

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Effect of Cimetidine on Levels of Some Oxidative Stress Biomarkers: Ameliorative Effect Of Vitamin C

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Abstract

Cimetidine is a drug widely used in the treatment of dyspepsia. The aim of this study was to evaluate the effect of chronic cimetidine treatment at therapeutic dose on levels of some oxidative stress biomarkers and possible ameliorative effect of vitamin C on any changes induced by cimetidine treatment. Fourty 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 each animal was anaesthetized and blood was collected by cardiac puncture. Serum malonyl dialdehyde (MDA), whole blood superoxide dismutase (SOD) and erythrocyte SOD levels were determined using commercially available kits. Cimetidine did not significantly ($P > 0.05$) affect serum MDA ($2.77 \pm 0.10 \mu\text{M}$ in control and $3.15 \pm 0.16 \mu\text{M}$ in cimetidine treated animals); whole blood SOD ($56.23 \pm 3.74 \text{ U/ml}$ in control and $63.30 \pm 4.24 \text{ U/ml}$ in cimetidine treated animals) and erythrocyte SOD ($393.66 \pm 28.06 \text{ U/g Hb}$ in control and $453.52 \pm 31.09 \text{ U/g Hb}$ in cimetidine treated animals). It was concluded that chronic treatment with cimetidine at therapeutic dose was not associated with lipid peroxidation or depletion of SOD. However, co-administration with vitamin C reversed the insignificant increase in the level of oxidative stress biomarkers.

Keywords: *cimetidine, malonyl dialdehyde, superoxide dismutase and vitamin C.*

Introduction

Cimetidine is a potent H₂-receptor antagonist [1] which is used in the treatment of dyspepsia. Due to the high prevalence of dyspepsia reported to be between 23-45% where Spain had 23.9% [2], US 32% [3] with significantly higher rates of 38-41% in the UK [4] and 45% in Nigeria [5], cimetidine is widely prescribed worldwide [6] but it is also available without [7,8]. Treatment is for a period of 4–8 weeks even if symptomatic relief has been achieved sooner and may also be continued for longer periods in those patients who may benefit from reduction of gastric secretion [9].

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses [10]. Significant disturbances in the normal redox state of cells can cause toxic effects through the production of free radicals that damage all components of the cell, including proteins, lipids, and DNA [11,12]. However low levels of ROS do not cause hazardous

events such as lipid peroxidation and oxidative DNA damage but play a physiologically important role in gene expression, cellular growth, defence against infection and modulation of messengers that regulate essential functions of cell membrane which are vital for survival [13,14]. Oxidative stress occurs only when free radicals are too numerous for antioxidants to adequately neutralize. Environmental pollutants, toxins, medications, infections, poor diet and radiation are all factors that contribute to the likelihood of this free radical/antioxidant imbalance [15]. An atom usually has a central nucleus with pairs of electron orbiting around it. However, when an atom or molecule has one or more unpaired electron in its outer shell it is called a free radical [16,17,18]. Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron; by cleavage of a radical to give another radical and lastly via redox reactions [11,12]. These free radicals are unstable and highly reactive molecules and so seek out to capture electrons from molecules in healthy cells to stabilize themselves.

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This leaves the molecules they stole an electron from destabilized and those molecules in turn seek out stabilization, leading to a chain of thousands of free radical reactions [15]. In addition some free radical species are converted to molecular oxidants like hydrogen peroxide, peroxyxynitrite and hypochlorous acid which in turn also act as a source of reactive oxygen species [19]. ROS and RNS are general terms collectively describing free radicals and other non-radical reactive derivatives also called molecular oxidants or molecular species [13]. Radicals are less stable but highly reactive compared to non-radical species. Under normal conditions formation of ROS and RNS can occur in the cells by two ways: enzymatic and non-enzymatic reactions.

Vitamin C is a potent antioxidant [20]. As an antioxidant, it protects the body from the harmful effects of free radicals [21,22]. It functions as an essential independent cellular antioxidant even in the presence of a vast excess of glutathione under oxidative challenge [23]. It is a very potent scavenger of superoxide and other reactive oxygen species [24]. It also protects against endogenous oxidative DNA damage [25].

Although toxicities related to drug-induced oxidative stress occur in multiple tissues [26] and oxidative stress plays an important role in the pathogenesis of numerous chronic-related free radical-induced diseases, it is not known whether oxidative stress has any role in the cimetidine – induced effects and whether or not the potent antioxidant vitamin C may be a modulator of this mechanism.

The aim of this study is to evaluate the effect of cimetidine on serum MDA, whole blood SOD and erythrocyte SOD of the control and cimetidine-treated rats and to determine any modulatory role of vitamin C on changes caused by cimetidine treatment in the above parameters.

Materials

Animals and Conditions

A total of forty adult male Wistar rats weighing 120 ± 10 grams, 90 days old [27] were obtained from the animal house of the Department of Human physiology, Bayero University, Kano, where the study was carried out. They were housed in plastic

cages with plastic bottom and maintained under adequate ventilation in the animal house. They were also allowed access to standard laboratory rat chaw and tap water *ad libitum* throughout the study. Experimental protocols were in accordance with the guidelines for animal research, as stated in the NIH Guidelines for the Care and Use of Laboratory Animals [28].

Preparation of Administered Drugs

The following drugs were used for the study:

1. Cimetidine 200mg tablets (Sam Pharmaceuticals, Nigeria) obtained from Well Care Pharmacy, Kano, Nigeria.

2. Pure grade vitamin C powder (Bulk supplements, Nevada, U.S.A.) obtained from Bridge Scientific laboratory, Ilorin, Nigeria.

Five tablets (1000mg) of cimetidine were crushed into powder using pestle and mortar and dissolved in 100mls of distilled water to make a stock solution containing cimetidine, at a concentration of 10 mg ml⁻¹. This was stored at room temperature, protected from light and used up within 3 days. Similarly, a stock solution containing vitamin C at a concentration of 10 mg ml⁻¹ was prepared and also stored at room temperature, protected from light and used within 3 days.

Methods

Experimental Design and grouping

A total of 40 rats were used for the study. They were randomly divided into 4 groups of 10 rats each. Each animal was administered either drugs or distilled water orally using metallic canula at 10:00am daily for 60 days.

Group 1 (Control): Distilled water

Group 2: Cimetidine at a therapeutic dose of 30 mg kg⁻¹ daily

Group 3: Cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ daily

Group 4: Cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ daily

Drug dosages used were described by [8]. The rats were weighed at the beginning of the experiment and every week subsequently. Doses of administered drugs were adjusted as appropriate to the weight of the animals.

Animals Sacrifice and Samples Collection

At the end of the experiment each rat was anaesthetized by chloroform inhalation in a gas chamber. The chest was then opened and blood samples aspirated by cardiac puncture using 10ml syringe. Blood collected was quickly discharged into plain bottles for determination of oxidative stress biomarkers [29].

MDA Determination

This was determined using Colorimetric TBARS microplate assay kit (Oxford Biomedical Research, Inc, U.S.A., Product code: FR40). The principle of this method is described as follows; 2-Thiobarbituric acid reactive substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress [30]. TBARS assay are reported in MDA equivalents. Although traditional TBARS assay has been criticized for its reactivity towards other compounds other than MDA [31], this kit gives a straight forward, reproducible and consistent method for analyzing biological samples for lipid peroxidation products and is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm. The procedure was carried out as follows;

1. 100 µl of saturated solution of ammonium sulfate was added to 0.5 ml of each serum in a microcentrifuge tube.
2. 35 mg of trichloroacetic acid (TCA) was added to each sample and vortexed.
3. The tubes were then centrifuged and the supernatant was transferred to clean tubes in duplicate and was tagged as sample.
4. Standards, samples and blanks were then prepared as follows; 200 µl of indicator solution was added to 200 µl of each standard and sample and were called standards and samples, respectively. 200 µl of acid reagent was added to the other duplicate samples and were referred to as sample blanks.
5. The standards, samples and blanks were heated at 65°C for 45 minutes.

6. 150 µl of each solution was transferred to a microplate and read at 540 nm.
7. The absorbance of each sample blank was subtracted from sample absorbance.
8. A standard curve was plotted with absorbance on y-axis and concentration of standards on x-axis.
9. MDA concentration for each sample was calculated using equation generated from standard curve. The result was multiplied by the dilution factor of 1.2.

Whole Blood SOD Determination

This was determined using SOD kit (Fortress diagnostics, Antrim, U.K., Product code: BXC0531). The test principle is based on the role of SOD to accelerate the dismutation of the toxic superoxide radical, produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. Fortress method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T. under the conditions of the assay. The procedure was carried out as follows:

1. Contents of R2 (buffer) was used to reconstitute R1 (mixed substrate).
2. Standard S6 (R4) was reconstituted with 10 mls of distilled water. Other standards (S2-S5) were prepared by serial dilution from S6 using sample diluent R5. Standard S1 was already reconstituted by the manufacturers.
3. 0.5 ml of each whole blood sample in EDTA bottles were centrifuged for 10 minutes at 3500 revolutions per minute and the plasma was aspirated. The red cells were washed four times with 0.9% saline and each time they were centrifuged.
4. The washed centrifuged red cells were then made up to 2 ml with cold distilled water, mixed and left to stand at 4°C for 15 minutes. This was now called diluted sample.
5. Sample diluent was prepared by mixing 50 µl of sample diluent R6 with 1.7 ml of mixed substrate R1.

6. Standards (S2-S6) were prepared by mixing 50 μ l of each standard R4 (S2-S6) with 1.7 ml of mixed substrate R1.
7. Diluted samples were prepared by mixing 50 μ l of each diluted sample with 1.7 ml of mixed substrate R1.
8. All the above preparations were mixed well and to each were added 250 μ l of Xanthine Oxidase (R3) and mixed again, incubated for 30 seconds at 37°C.
9. Once the incubation time reached 30 seconds the first reading (absorbance) was taken using a spectrophotometer. Readings were taken again at 1, 2 and 3 minutes. The mean absorbance change per minute was determined and used to determine the percentage inhibition.
10. A graph of percentage inhibition for each standard against Log_{10} (standard concentration in SOD units/ml) was plotted.
11. The percentage inhibition of each sample was used to obtain units of SOD from standard curve.
12. Results obtained from the standard curve were multiplied by 100 (dilution factor).

Erythrocyte SOD Determination

This was obtained by using the following formula stated in the SOD kit (Fortress diagnostics, Antrim, U.K., Product code: BXC0531)

$$\text{Erythrocyte SOD} = \frac{\text{Whole blood SOD}}{\text{Hb g/ml}}$$

Statistical Analysis

All data were presented as mean $\pm$ SEM and were analyzed using SPSS version 20.0 (SPSS Inc, Chicago, United States). ANOVA and Tukey post-hoc tests were used to compare means. Values of $P \leq 0.05$ were considered significant.

Results

Serum MDA (μ M)

Figure 1 shows the levels of serum MDA of rats following 60 days of treatments. There was a significant difference between the groups in serum MDA levels ($P = 0.001$). Serum MDA levels of cimetidine 30 mg kg^{-1} group (3.15 ± 0.16) was not significantly different from control group (2.77 ± 0.10) ($P = 0.593$). This shows that serum MDA level was not increased by 30 mg kg^{-1} cimetidine. Serum MDA levels of cimetidine 30 mg kg^{-1} + vitamin C 25 mg kg^{-1} group (1.52 ± 0.30) and cimetidine 30

mg kg^{-1} + vitamin C 50 mg kg^{-1} group (1.24 ± 0.23) were significantly different compared to control group (2.77 ± 0.10) ($P = 0.001$). This suggests that vitamin C at both doses decreased serum MDA levels. There was no difference in serum MDA levels of cimetidine 30 mg kg^{-1} + vitamin C 50 mg kg^{-1} group (1.24 ± 0.23) when compared to cimetidine 30 mg kg^{-1} + vitamin C 25 mg kg^{-1} group (1.52 ± 0.30) ($P = 0.783$). This indicates that the effect of vitamin C on this parameter is not dose dependent. There was a significant difference in serum MDA levels of cimetidine 30 mg kg^{-1} + vitamin C 25 mg kg^{-1} group (1.52 ± 0.30) and cimetidine 30 mg kg^{-1} + vitamin C 50 mg kg^{-1} group (1.24 ± 0.23) compared to cimetidine 30 mg kg^{-1} group (3.15 ± 0.16) ($P = 0.001$). This shows that both doses of vitamin C decreased serum MDA levels significantly when compared to 30 mg kg^{-1} cimetidine.

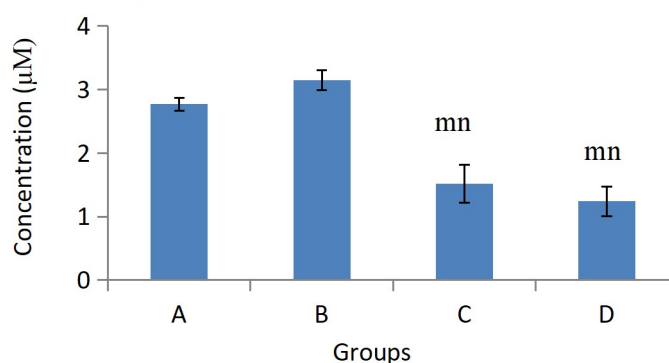


Figure 1: Levels of serum MDA of rats following 60 days of treatments (Mean $\pm$ S.E.M., $n = 10$)

A = Group 1 (Control): Distilled water
 B = Group 2: Cimetidine at a therapeutic dose of 30 mg kg⁻¹ daily
 C = Group 3: Cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ daily
 D = Group 4: Cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ daily
^m = significant compared to control group
ⁿ = significant compared to cimetidine 30 mg/kg group

Whole blood SOD (U/ml)

Figure 2 shows the levels of whole blood SOD of rats following 60 days of treatments. There was a significant difference between the groups in whole blood SOD levels ($P = 0.001$). Whole blood SOD levels of cimetidine 30 mg kg⁻¹ group (63.30 ± 4.24) was not significantly different from control group (56.23 ± 3.74) ($P = 0.823$). This shows that whole blood SOD level was not decreased by treatment with 30 mg kg⁻¹ cimetidine. Whole blood SOD levels of cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹

group (100.28 ± 9.16) and cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ group (97.92 ± 4.23) were significantly higher compared to control group (56.23 ± 3.74) ($P = 0.001$). This suggests that cimetidine co-administered with vitamin C at both doses increased whole blood SOD levels. There was no difference in whole blood SOD levels of cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ group (97.92 ± 4.23) when compared to cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ group (100.28 ± 9.16) ($P = 0.991$). This indicates that the effect of vitamin C on this parameter is not dose dependent. There was a significant difference in whole blood SOD levels of cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ group (100.28 ± 9.16) and cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ group (97.92 ± 4.23) compared to cimetidine 30 mg kg⁻¹ group (63.30 ± 4.24) ($P = 0.001$). This shows that both doses of vitamin C increased whole blood SOD levels significantly when compared to 30 mg kg⁻¹ cimetidine.

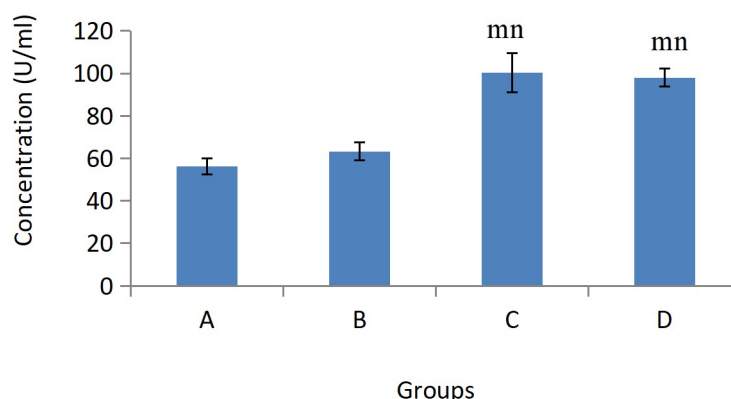


Figure 2: Levels of whole blood SOD of rats following 60 days of treatments (Mean ± S.E.M., $n = 10$)

A = Group 1 (Control): Distilled water
 B = Group 2: Cimetidine at a therapeutic dose of 30 mg kg⁻¹ daily
 C = Group 3: Cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ daily
 D = Group 4: Cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ daily
^m = significant compared to control group
ⁿ = significant compared to cimetidine 30 mg/kg group

Erythrocyte SOD (U/g Hb)

Figure 3 shows the levels of erythrocyte SOD of rats following 60 days of treatments. There was a significant difference between the groups in erythrocyte SOD levels ($P = 0.001$). Erythrocyte SOD levels of cimetidine 30 mg kg⁻¹ group (453.52 ± 31.09) was not significantly different from control group (393.66 ± 28.06) ($P = 0.725$). This shows that erythrocyte SOD level was not decreased by treatment with 30 mg kg⁻¹ cimetidine. Erythrocyte SOD levels of cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ group (696.51 ± 62.61) and cimetidine 30

mg kg⁻¹ + vitamin C 50 mg kg⁻¹ group (676.64 ± 29.88) were significantly higher compared to control group (393.66 ± 28.06) ($P = 0.001$). This suggests that co-administration of cimetidine and vitamin C at both doses increased erythrocyte SOD levels. There was no difference in erythrocyte SOD levels of cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ group (676.64 ± 29.88) when compared to cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ group (696.51 ± 62.61) ($P = 0.985$). This indicates that the effect of vitamin C on this parameter is not dose dependent. There was a significant difference in erythrocyte

SOD levels of cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ group (696.51 ± 62.61) and cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ group (676.64 ± 29.88) compared to cimetidine 30 mg kg⁻¹ group (453.52 ± 31.09) ($P = 0.001$ and $P = 0.002$, respectively). This shows that both doses of vitamin C increased erythrocyte blood SOD levels significantly when compared to 30 mg kg⁻¹ cimetidine.

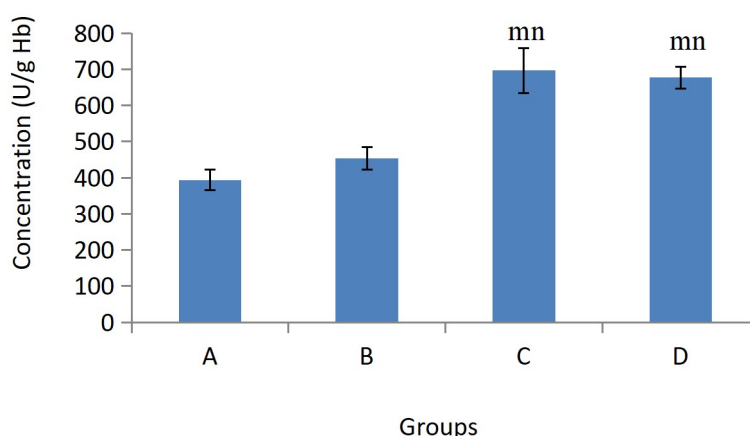


Figure 3: Levels of erythrocyte SOD of rats following 60 days of treatments (Mean ± S.E.M., n = 10)

A = Group 1 (Control): Distilled water

B = Group 2: Cimetidine at a therapeutic dose of 30 mg kg⁻¹ daily

C = Group 3: Cimetidine 30 mg kg⁻¹ + vitamin C 25 mg kg⁻¹ daily

D = Group 4: Cimetidine 30 mg kg⁻¹ + vitamin C 50 mg kg⁻¹ daily

^m = significant compared to control group

ⁿ = significant compared to cimetidine 30 mg/kg group

Discussion

Cimetidine is clinically a very important drug, widely prescribed but also available without prescription [32]. It is commonly used in the treatment of gastric and duodenal ulcers [8]. Previous works on cimetidine were carried out using both acute and chronic high doses of the drug to demonstrate its effect, however little is known about such effects using the drug at therapeutic doses [33].

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants [10]. Free radicals may result in damage to some constituents of the body because the body is not necessarily fully protected against oxidative damage. The resultant oxidative products formed following the oxidative damage are referred to as biomarkers [16]. These substances can in turn be used to assess the level of oxidative stress. The accurate assessment of such stress is vital to investigate various pathological conditions and also to evaluate the efficacy of drugs. These markers found in blood, urine and other biological fluid may provide information of diagnostic value. Many markers have been proposed and they include lipid peroxides, malondialdehyde and 4-hydroxynonenal as markers for oxidative damage to lipids; carbonyl protein, hydrovaline, hydroxyleucine and nitrotyrosine for oxidative damage to protein and amino acids; 8-oxoguanine (8-hydroxyguanine) and thymineglycol as indicators of oxidative damage to DNA; isoprostane as a

product of the free radical oxidation of arachidonic acid [16].

Vitamin C, also known as ascorbic acid is a water-soluble vitamin. It is an organic compound that is necessary for normal physiological functions in the human body. Unlike most mammals and other animals, humans do not have the ability to make ascorbic acid and must therefore obtain it from diet. Vitamin C is a potent reducing agent [20], meaning that it readily donates electrons to recipient molecules. Related to these oxidation-reduction (redox) potential, two major functions of vitamin C are as an antioxidant and as an enzyme cofactor [34,35]. Of the two chemically distinct forms of vitamin C, only the reduced form ascorbic acid is present in human plasma cells and tissues, and the cellular concentration of this vitamin can exceed the plasma level $60 \mu\text{mol L}^{-1}$ by several orders of magnitude [36].

Cimetidine has caused an insignificant increase in serum MDA levels. This demonstrates that cimetidine did not significantly increase oxidative stress in the animals. Treatment with vitamin C at 25 mg kg^{-1} and 50 mg kg^{-1} resulted in a significant decrease in serum MDA levels. This shows improvement in antioxidant status by vitamin C. The mechanism of this effect may be due to the role of vitamin C as an antioxidant. It was reported that the overall consequence of supplementation of vitamin C is the beneficial control of lipid peroxidation therefore leading to a significant reduction in MDA levels which is one of the products of lipid peroxidation [37]. This finding is in agreement with that of [38,39,40] who reported a significant decrease in serum MDA levels following supplementation with vitamin C while [41,42] reported the amelioration of oxidative damage following the use of vitamin C.

Cimetidine has caused an insignificant increase in whole blood SOD levels. This demonstrates that cimetidine did not significantly increase oxidative stress in the animals. Treatment with vitamin C at 25 mg kg^{-1} and 50 mg kg^{-1} resulted in a significant increase in whole blood SOD levels. This indicates an improvement in the antioxidant status and a reduction in oxidative stress level. The mechanism of this effect may be due to the role of vitamin C as an antioxidant which results in boosting the activity

of endogenous antioxidant defenses to prevent the formation and oppose the actions of free radicals therefore maintaining health [43,44]. This study is similar to that of [39,45] who reported a significant increase in SOD concentration following vitamin C supplementation.

Cimetidine has caused an insignificant increase in erythrocyte SOD levels. This demonstrates that cimetidine did not significantly increase oxidative stress in erythrocytes of the animals. Treatment with vitamin C at 25 mg kg^{-1} and 50 mg kg^{-1} resulted in a significant increase in erythrocyte blood SOD levels. This mechanism of effect is due to the antioxidant effect of vitamin C and indicates an improvement in the protection of the biological integrity of the cells and tissues against the harmful effects of superoxide ions [46,47]. SOD catalyzes the dismutation (destruction) of superoxide free radical ions which are believed to cause the lipid peroxidation and peroxidative hemolysis of erythrocyte [47]. The finding of this study is in agreement with those of [40,45,48] who reported a significant increase in erythrocyte SOD concentration following vitamin C supplementation.

Conclusions

Cimetidine did not significantly affect serum MDA ($2.77 \pm 0.10 \mu\text{M}$ in control and $3.15 \pm 0.16 \mu\text{M}$ in cimetidine treated animals); whole blood SOD ($56.23 \pm 3.74 \text{ U/ml}$ in control and $63.30 \pm 4.24 \text{ U/ml}$ in cimetidine treated animals) and erythrocyte SOD ($393.66 \pm 28.06 \text{ U/g Hb}$ in control and $453.52 \pm 31.09 \text{ U/g Hb}$ in cimetidine treated animals). This shows that chronic treatment with cimetidine at therapeutic doses was not associated with lipid peroxidation or depletion of SOD, however, co-administration with vitamin C reversed the insignificant increase.

Recommendations

1. Vitamin C at 25 mg kg^{-1} may be recommended as co-treatment therapy with cimetidine especially in chronic conditions.
2. Further research on therapeutic doses of cimetidine should include co-administration with lower doses of vitamin C and assessment of oxidative stress in testicular tissues by determination of MDA level in testicular

homogenates and other biomarkers of oxidative stress.

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Assesment of Nutritional Knowledge, Attitude and Practices among Hypertensive Patients Attending Selected Hospitals in Kano Metropolis, Nigeria

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Abstract

Non-communicable diseases (NCDs) such as hypertension and diabetes are a major health burden in developed countries, and are on a rising alarm in developing countries owing to changes in demographic set-up due to high rate of urbanization which lead to dynamic change in lifestyles among people. The role of dietary approaches in the treatment of hypertension have been well documented. The aim of this study is to assess the nutritional knowledge, attitude and practices among hypertensive out-patients attending some hospitals in Kano metropolis. It was a cross-sectional study involving 200 participants (140 hypertensive and 60 non-hypertensive). Data on knowledge, attitude and practice (KAP) of nutrition were collected using a standard questionnaire. Nutritional attitude of hypertensive and non-hypertensive respondent, Over 80% of the subjects lack nutrition knowledge. The finding showed that there was poor attitude to healthy eating habits among the hypertensive patient respondents. Poor nutrition KAP was observed among the study participants, hence, there is a need to integrate nutrition education in the management of hypertension. Cost-effective non-pharmacological strategies like good nutrition knowledge, attitude and practices in management of hypertension are particularly needed in Nigeria due to limited resources since they are shared with the concurrent burden of persistent communicable diseases.

Keywords: *Nutrition, Hypertension, Knowledge, Attitude, Practice*

Introduction

Hypertension becomes serious public health problems in many industrialized countries encountering epidemiological changes from communicable to non-communicable diseases. This changes has ascribed to urbanization, aging and behavioural risk factors such as sedentary lifestyle, unhealthy diet, excessive alcohol consumption, smoking and chronic stress [1]. Non-communicable diseases or chronic conditions lead to long term demands on health care systems in which the conditions need on-going management over many years which leads to the poor management of the health system [2]. It has been estimated globally that 26% of all adults have hypertension [1]. Hypertension affects about 1.1 billion people worldwide and its prevalence is expected to increase by 60% by the year 2025. It is estimated that while LMICs bear 78% of all deaths from non-communicable diseases, they are also hard-hit with consequences of undernutrition [3]. In Nigeria the prevalence of hypertension in adults above 18 years

was 47.2% with the male having 13.9% and female with 12.7% [4].

Nutritional status results from the relationship between nutrient intake and requirements; and the body's ability to digest, absorb and use these nutrients [5]. Nutritional status is optimal when nutritional intake and needs are equal. A deviation from this results in malnutrition, a pathological condition caused by unbalanced or insufficient diet or defective assimilation and utilization of foods. Malnutrition refers to all deviations from adequate nutrition, which can be due to inadequacy of food (under nutrition) resulting in protein energy malnutrition, micronutrient deficiencies etc; or due to excess food intake (overnutrition) resulting in metabolic disorders such as obesity, diabetes and cardiovascular diseases. Thus, a poor nutrition reduces immunity, increases susceptibility to disease, impairs physical and mental development, and reduces productivity [6]. Inadequate diet and physical inactivity causes overweight and obesity, both of

which are responsible for the significant increase in hypertension worldwide, especially in adolescents [7]. Also, the restriction of vital nutrients during pregnancy leads to intrauterine growth restriction on organs and their functions, resulting in low birth weight and placental size, both of which contribute to the development of hypertension and other chronic diseases in adulthood [8].

Materials and Methods

Study Participants

The study was an observational, cross-sectional study. The study participants comprise of hypertensive patients and apparently healthy persons between the ages of 20 and 70 years. Hypertensive participants were selected by simple random probability sampling method from patients (males and females) attending the hypertensive clinic of selected hospitals in Kano metropolis (Murtala Muhammad Specialist Hospital, Sheik Muhammad Jidda Hospital, Sir Muhammad Sunusi General Hospital and Muhammad Abdullahi Wase Specialist Hospital), Kano State, Nigeria.

Inclusion and Exclusion Criteria

Patients included in the study were those that have been diagnosed as hypertensive. The hypertensive status of the patients was defined as described by the United State Seventh Joint National Committee on Detection, Evaluation and Treatment of hypertension (JNC-VII), that is, systolic blood pressure greater than or equal to 140 mmHg and diastolic blood pressure greater than or equal to 90 mmHg. Women receiving oral contraceptives or hormone replacement therapy, pregnant women and lactating

mothers, as well as those that refused to give their consent were excluded from the study.

Ethical Approval

Ethical approval was obtained from Kano State Hospital Management Board (HMB) (MOH/Off/797/T.I/1649). Participants were duly informed about the research and gave informed consent. All information were kept confidential.

Data collection

Following informed consent, a structured interviewer-administered questionnaire was used to elicit data on subjects socioeconomic and demographic characteristics, Nutritional knowledge, attitude and practice (KAP), age, sex, marital status, occupation and educational level attained, monthly income and number of children.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL). Numerical data was expressed as mean and standard deviation or median and range as appropriate. Qualitative data was expressed as frequency and percentage. Chi-square test, confidential interval and odd ratio were used to examine the association between qualitative variables.

Results

Table 1 shows the socio-demographic data of the study participants. Among the 200 participants (both hypertensive and normotensive), 128 (69%) were female and 72 (31%) were male, majority of the participant (80%) were within the age range of 45-70 years among which 64% were hypertensive.

Table 1: *Socio-Demographic Characteristics of Hypertensive and Normotensive Participants.*

	Total (n=200)n(%)	Hypertensive (n =160) n(%)	Normotensive (n=40) n (%)	Chi²	P
SEX					
Male	72(36)	56 (28)	16 (8)	0.347	0.556
Female	128(64)	104 (52)	24 (12)		
AGE					
25-34	16(8)	8 (4)	8 (4)	15	0.001
35-44	24(12)	24 (12)	0 (0)		
45-70	160(80)	128 (64)	32 (16)		

MARITAL STATUS

Married	184(92)	152 (76)	32 (16)	9.783	0.002
Divorced	16(8)	8 (4)	8 (4)		

EDUCATIONAL LEVEL

No formal Education	44(22)	32 (16)	12 (6)	3.989	0.136
Secondary	116(58)	92 (46)	24 (12)		
Tertiary	40(20)	36 (18)	4 (2)		

EMPLOMENT STATUS

Self employed	64(32)	48 (24)	16 (8)	1.842	0.398
Civil Servant	76(38)	64 (32)	12 (6)		
Not Employed	60(30)	48 (24)	12 (6)		

MONTHLY INCOME

<#18,000	80(40)	68 (34)	12 (6)	6.042	0.11
#18 –#50,000	24(12)	16 (8)	8 (4)		
#51 – #100,000	64(32)	48 (24)	16 (8)		
>#100,000	32(16)	28 (14)	4 (2)		

NUMBER OF CHILDREN

None	63(31.5)	63 (31.5)	0 (0)	31.245	0.001
1 – 4	13(6.5)	9 (4.5)	4 (2)		
5 – 8	44(22)	28 (14)	20 (10)		
9 – above	76(38)	60 (30)	16 (8)		

Table 2 indicates the nutritional knowledge of the hypertensive and control individuals attending some selected hospitals in Kano metropolis. Lack of nutritional knowledge among the participants was observed.

Table 2: Nutritional Knowledge among the Hypertensive and Control Individuals Attending Selected Hospitals in Kano Metropolis

QUESTIONS	CHOICE	HYP(%)	CONT(%)	X ²
Awareness on significance of nutrition	YES	77.8	22.2	0.713
	NO	80.5	19.5	
Awareness on balance diet	YES	75.0	25.0	0.440
	NO	81.0	19.0	
Awareness on main food nutrients	YES	71.4	28.6	0.059
	NO	83.3	16.7	
Awareness on the risk of overweight	YES	66.7	33.3	0.002
	NO	85.7	14.3	
Awareness on the knowledge of food for growth and development	YES	69.2	30.8	0.024
	NO	83.8	16.2	
Awareness on the risk of overfeeding	YES	66.7	33.3	0.002
	NO	85.7	14.3	
Awareness on harmful foods	YES	73.3	26.7	0.123
	NO	82.9	17.1	
Awareness on the best source of nutrition	YES	76.2	23.8	0.252

information is hospitals	NO	82.8	17.2	0.247
Having problems with appetite	YES	78.6	21.4	
	NO	87.5	12.5	0.123
Loosing weight using pills, laxatives, not eating	YES	82.9	17.1	
	NO	73.3	26.7	

HYP (Hypertensive), CONT (non-hypertensive)

Table 3 shows the result of attitude and practice among the participant in which the larger percentage of the population were found to practice consumption of foods rich in carbohydrate (78%). Most of them do not practice to plan and keep to a balance food menu (64%). Almost all the participants rarely practice eating foods rich in fats and oils.

Table 3: Attitude and Practice among Hypertensive and Control Individual Attending Selected Hospitals in Kano Metropolis

QUESTIONS	CHOICE	HYP (%)	CONT (%)	X ²
How often do you eat food substance rich in carbohydrate	PA	76.9	23.1	0.041
	DP	0.0	0.0	
	PR	90.9	9.1	
How often do you eat body building foods like egg, beans and milk	PA	77.8	22.2	0.556
	DP	0.0	0.0	
	PR	81.2	18.8	
How often do you eat fats and oils food substances	PA	50.0	50.0	0.030
	DP	0.0	0.0	
	PR	81.2	18.8	
How often do you eat food that are rich in vitamins like fruits,vegetable etc.	PA	80.0	20.0	0.039
	DP	88.9	11.1	
	PR	72.7	27.3	
How often do you drink eight and more glasses of water daily	PA	87.0	13.0	0.023
	DP	0.0	0.0	
	PR	74.1	25.9	
How often do you plan and keep to a balance food menu	PA	66.7	33.3	0.000
	DP	75.0	25.0	
	PR	100	0.0	
How often do you eat foods rich in fibre/roughages like skin of fruits, wheat and grains	PA	86.7	13.3	0.264
	DP	80.0	20.0	
	PR	76.0	15.4	
How often do you eat fried foods and other fattening foods	PA	50.0	50.0	0.006
	DP	80.0	20.0	
	PR	84.6	15.4	
How often did you exercise	PA	83.3	16.7	0.353
	DP	75.0	25.0	
	PR	83.3	16.7	
How often do you take vitamins and minerals supplements	PA	50.0	50.0	0.000
	DP	70.4	29.6	
	PR	95.2	4.8	

PA= Practice Always DP= Do not Practice PR= Practice Rarely

Discussion

This study was carried out in the general Hospitals located at the urban area of Kano state, in which the participants attending the hospitals are generally from poor background because health service in the urban area are averagely costly as such the poor people prepare government hospitals. Most of the participants were married women with the highest percentage having primary education.

Nutritional knowledge of the hypertensive and normotensive individuals attending selected hospitals in Kano metropolis in which more than 80% of the hypertensive respondents are unaware of the significance of nutrition, balance diet, main sources of food nutrient, risk of obesity and the best sources of nutrition information is hospitals, also 80 % of the hypertensive individuals are aware on the use of pills, laxatives for weight lost and not food. So also the non-hypertensive participants that served as the control group indicated their overall unawareness on nutritional knowledge. The results show significant difference ($p < 0.005$) between the two groups on awareness on the risk of overweight and awareness on the risk of overfeeding. In general there is lack of nutritional knowledge among the participants.

In terms of attitude and practice most of the participants always practice consumption of food rich in carbohydrate. The finding could be related with that of a similar study which states that sound educational attainment is directly proportional to the nutritional knowledge, attitude and practice [9]. Poor nutritional knowledge, attitude and practices are directly related to socio-economic factors, poverty, ignorance and poor educational background in a society with inadequate formal education and high risk of hypertension and other diseases [9].

Conclusion

Poor nutrition knowledge, attitude and practice was observed among the study participants, hence, there is high need to integrate nutrition education interventions aimed at improving nutritional knowledge, attitude and practices should be enforced focusing on the prevention and management of hypertension

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