

# **Bayero Journal of Biomedical Sciences (BJBS)**

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- ❖ Recent research <sup>[1]</sup> reported the value of DNA markers in this respect.
- ❖ Several studies have reported the increased prevalence of cancer. <sup>[2-5, 8]</sup>

- ❖ The analysis was carried out according to the method reported by Muhammad *et al.* <sup>[12]</sup>

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4. Web page/website: World Health Organization. Global diabetes compact. Available from: [www.who.int/news/global-diabetes-compact](http://www.who.int/news/global-diabetes-compact) (accessed 12<sup>th</sup> 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 5, 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 Due to so many logistic problems, the biannual publication has not been archived for the past three year. 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 on Covid-19 global pandemic, 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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## Acute Effects of Caffeine Administration on Cognitive and Cardiovascular Parameters among Undergraduate Students of Bayero University, Kano

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### Abstract

Caffeine is a bitter alkaloid that is commonly consumed in form of coffee, tea, energy drinks and chocolates. Its consumption in Nigeria is currently on the rise, especially among University students. Previous studies have reported varying results in the observed effects of caffeine on cognitive and cardiovascular functions. This study evaluated the short-term effect of caffeine ingestion on cognition and cardiovascular parameters amongst students of Bayero University Kano. Fifty (50) apparently healthy volunteers (aged 20 – 27years) were recruited following informed consent and adherence to approved ethics for human research. Cognitive function was assessed using Montreal cognitive assessment tool, whereas cardiovascular parameters such as heart rate, systolic and diastolic blood pressures were determined 1,440 minutes before and 60 minutes after acute caffeine (3 mg/kg) administration. The subject's attention, memory, stroop test, visual recall and reaction time were significantly increased following acute caffeine intake. The visuospatial function of the participants before caffeine administration was only slightly improved. Similarly, caffeine intake was found to significantly increase systolic blood pressure and heart rate, but had no effect on diastolic blood pressure. Thus, acute caffeine intake could improve cognitive function and may exert a moderate rise in cardiovascular parameters.

**Keywords:** Caffeine, Cognition, Cardiovascular parameters

### Introduction

Caffeine is a bitter, crystalline-white methyl xanthine alkaloid that is structurally related to adenosine and acts as a central nervous system (CNS) stimulant.<sup>[1,2]</sup> It is commonly consumed in the form of coffee, tea, energy drinks and chocolates as a potent stimulant and cognition enhancer.<sup>[3-9]</sup> There is an enormous rise in the global consumption of caffeine, with Nigeria recording more than 20% rise at the interval of 2010 to 2015.<sup>[10,11]</sup>

Similarly, over the last few decades, caffeine intake has risen significantly among university students.<sup>[12]</sup> Students rely on caffeine as a means of sleep deprivation and a source of energy.<sup>[13]</sup> According to Malinauskas *et al.*<sup>[13]</sup> caffeine is consumed at relatively dangerous levels, 83% of student consumers have shown signs of intoxication and another 51% showed signs of withdrawal.

Caffeine has so far been termed the most frequently ingested pharmacological active substance worldwide.<sup>[6]</sup> Memory, attention, perception, language and psychomotor functions form components of cognitive functions that could be affected by the amount or frequency of caffeine

consumption.<sup>[14]</sup> Similarly, some cardiovascular parameters such as heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) can also be affected by caffeine consumption.<sup>[15,16]</sup> However, body responses to acute caffeine administration may vary between populations due to compounding metabolic and pathologic factors.<sup>[17]</sup> This study was designed to evaluate the effect of caffeine on executive functions, memory, attention, as well as cardiovascular functions among students of Bayero University, Kano-Nigeria.

### Methods

#### Inclusion and Exclusion Criteria

Before the study, volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers, individuals with a history of heart conditions and/or allergies to caffeine were excluded from the study.



## Participants

Fifty apparently healthy adult volunteers (17 males and 33 females, age range 20 – 27 years) participated in the study. They were undergraduate students from the faculty of Basic Medical Sciences. Participants abstained from caffeine and alcohol for a minimum of 12 h before the first testing session and until the final testing session was completed. The study was approved by Health Research Ethics Committee, Ministry of Health, Kano State, Nigeria and was carried out in accordance with the Helsinki Declaration 1975, as revised in 2000 (available at [http://www.wma.net/e/policy/17-c\\_e.html](http://www.wma.net/e/policy/17-c_e.html)). The study was a repeated measure experimental design.

## Assessment

Participants received 3 mg/kg of caffeine in the morning (9:00 am), at exactly 24 hours after complete caffeine abstinence. Montreal cognitive assessment (MOCA) questionnaire was used to assess the subject's cognitive score 24 hours prior to-and 24 hours after- caffeine administration. Test practice trial was administered to familiarize the participants with the test. Short term memory of the participants was assessed by performing a recall test for a list of five words. Attention was determined using sequence analysis. The visual recall was assessed by displaying a list of ten alphabets at the rate of one alphabet per second.<sup>[5]</sup> Stroop Test was

conducted by displaying a list of differently coloured words.<sup>[5]</sup> Furthermore, reaction time was determined as previously described by Paulus and colleagues.<sup>[5]</sup>

The prepared caffeine solution was served in disposable cups and consumed by each participant. The participants were allowed to walk around the lab, talk and indulge in mild activities and after about 60 minutes, the test procedure was repeated again.<sup>[17]</sup> MOCA questionnaires were re-administered to the participants and subsequently, visual recall, stroop test and reaction time were determined. The systolic blood pressure, diastolic blood pressure and heart rate were recorded.

## Statistics

Data obtained was analyzed using SPSS version 22. Descriptive statistics were used to express mean and standard deviation. Paired sample t-test was used to find the mean, standard deviation and significant difference between pre and post caffeine values for all variables.

## Results

A total of 50 undergraduate students participated in the study. Table 1 shows that 66% of the participants were females and about 34% were males. The mean age and weight of the participants did not differ significantly between sexes.

**Table 1: Age and Weight Distribution of Participants**

| Variable | Male (Mean±SD) | Female (Mean±SD) | p-value | t-value |
|----------|----------------|------------------|---------|---------|
| Age      | 22.64±1.729    | 23.76±1.954      | 0.587   | -2.092  |
| Weight   | 58.35±9.069    | 53.88±10.809     | 0.787   | -1.461  |

## Effects of Caffeine on Cognitive Domains

Caffeine taken at a dose of 3 mg/kg of body weight was seen to cause an increase in visuospatial/executive function of the participants ( $4.28 \pm 0.730$ ). However, this improvement was not

statistically significant ( $p > 0.05$ ). Attention, memory, stroop test, visual recall and reaction time were all significantly increased ( $p < 0.05$ ) following caffeine administration (Table 2).

**Table 2: Effects of Acute Caffeine Administration on Cognitive Domains**

| Parameters    | pre-caffeine | post-caffeine | t-value | p-value |
|---------------|--------------|---------------|---------|---------|
| Attention     | 5.48±0.814   | 5.84±0.422*   | -3.280  | 0.002*  |
| Memory        | 4.56±0.705   | 4.92±0.340*   | -3.174  | 0.003*  |
| Stroop test   | 4.80±2.515   | 7.48±2.121*   | -9.178  | 0.000*  |
| Visual        | 6.48±1.502   | 7.12±1.438*   | -4.413  | 0.000*  |
| Reaction      | 13.06±11.750 | 17.34±8.856*  | -2.174  | 0.035*  |
| Executive fxn | 4.10±1.015   | 4.28±0.730    | -1.353  | 0.182   |

\*Statistically significant variation  $p \leq 0.05$ ). Data is presented as Mean ±SD

### Effects of caffeine on cardiovascular parameters

Caffeine taken at a dose of 3 mg/kg of body weight was seen to cause a significant increase ( $p>0.05$ ) in

SBP and HR but no significant effect ( $p<0.05$ ) was seen in DBP (Table 3).

**Table 3:** Effects of acute caffeine administration on cardiovascular parameters

| Parameters         | pre-caffeine  | post-caffeine | t-value | p-value |
|--------------------|---------------|---------------|---------|---------|
| Sys BP (mmHg)      | 116.40±10.451 | 121.80±8.965* | -3.623  | 0.001*  |
| Dias BP (mmHg)     | 80.20±10.593  | 79.80±8.919   | 0.280   | 0.780   |
| Heart Rate (b/min) | 77.32±8.886   | 84.48±9.298*  | -7.331  | 0.000*  |

\*Statistically significant variation ( $p\leq0.05$ ). Data is presented as Mean ±SD, BP: Blood Pressure Sys: Systolic, Dias: Diastolic.

### Discussion

The findings of this study indicate that caffeine administration significantly improves cognitive domains and functions. Although it increased visuospatial/executive function, its effect was not statistically relevant. This observation agrees with the findings of Pipijn *et al.*<sup>[18]</sup> who showed the significant effect of caffeine on executive function among participants tasked with several classical dual-processing paradigms and implicit recollection tests. According to Pipijn and colleagues,<sup>[18]</sup> caffeine had no effect on executive function. The sustenance of similar findings as regard to visuospatial/executive function is attributable to the similarity in test methodology. Furthermore, in both studies, the subject's cognitive functions were analyzed 60 min after caffeine intake. Contrary to our findings, Soar *et al.*,<sup>[19]</sup> reported a significant effect of caffeine across a number of executive functioning constructs. The study was conducted using a supposedly more sensitive tool, a non-immersive virtual reality assessment of executive functions known as JEF (©) (the Jansari Assessment of Executive function). The results of their works demonstrated the ability of JEF (©) to detect the effect of caffeine across a number of executive functioning constructs, which weren't shown in MOCA. Their findings showed that performance was significantly improved on planning, creative thinking, event-, time-, and action-based prospective memory.

The optimal level of performance was achieved with caffeine when comparing its effects on attention which was significantly improved. This can be supported by earlier findings reported by Nikic *et al.*<sup>[20]</sup> According to the authors, the attention

function of 67 subjects was assessed using MOCA questionnaire and a significant increase was observed among caffeine treated subjects. Previous works have been consistent with the fact that even small doses (<40mg) of caffeine can improve performance efficacy on attention task even when taken in delivery forms like chewing gum.<sup>[21]</sup> Though, another study carried out by Brunye *et al.*<sup>[22]</sup> showed that caffeine enhances attention but only at the highest administered doses (400mg). The result seen for all the previous researches is consistent with the findings of the present study. Most of these researches made emphasis on the doses administered to be the rate-limiting step of caffeine's effect on attention. The result of this study also indicated that caffeine caused a significant increase in memory. Similarly, Paulus *et al.*<sup>[5]</sup> noted a significant improvement in memory of subjects that were given caffeine relative to a vehicle following both auditory and visual recall test. However, contrary to our findings, Terry and Anthony showed that short term memory of rats was not augmented by caffeine administration.<sup>[23]</sup> However, specie variation could account for the difference in the effect of caffeine on short term—memory. In addition, caffeine intake significantly increased stroop test accuracy, which was consistent with the findings by Paulus *et al.*<sup>[5]</sup> and contradicts that of Dixit *et al.*<sup>[24]</sup> Furthermore, caffeine produced a significant ( $p<0.05$ ) increased in the visual recall. Paulus *et al.*<sup>[5]</sup> reported similar findings following random projection of ten letters to 60 college students who were administered caffeine contained in different vehicles.

The finding of this study further relates reaction time with caffeine intake. Caffeine increases the time required from the release of the ruler to its grasp by

the subject, therefore exerting a negative effect. A pilot study by Hickey (using almost similar dosing of caffeine) showed a significant increase in reaction time at 40 minutes post caffeine consumption.<sup>[25]</sup> The result could be attributed to the relatively close amount of caffeine administered (< 4 mg/kg) in both studies. Contrarily, a study conducted by Blunden reported an improvement in reaction time following administration of 5 mg/kg caffeine to taekwondo.<sup>[26]</sup>

Caffeine exhibited significant short term improvement on systolic blood pressure and heart rate, but no significant effect was seen on diastolic blood pressure. A study carried out by Geethavani *et al.*<sup>[27]</sup> stated that caffeine increases systolic blood pressure and HR as a consequence of increased stiffness of the aorta and larger blood vessels, rather than increased stroke volume. The study further states that the increased stiffness is attributed to increased production of angiotensin II, catecholamines and the inhibition of A2 receptors activity in smooth muscles. A contrary finding was reported by Kumar and Verma, wherein the blood pressures of young adults and geriatrics that habitually consume coffee were compared.<sup>[28]</sup> SBP and DBP were more elevated in young adults than the elderly, whereas HR was significantly reduced in both groups. Few other studies also revealed that caffeine increases SBP,<sup>[29,30]</sup> while a few others showed no significant effect on BP and HR.<sup>[31]</sup>

Therefore it can be concluded from this study that acute caffeine intake improves cognitive function such as attention, short term memory, visual recall, executive/visuospatial functions and stroop effect. However, further examination of long term effects of caffeine on cognition, the use of larger sample size and application of more sensitive methods to analyze parameters such as reaction time may provide a better understanding of caffeine's effect on cognitive function. In addition, caffeine increases systolic blood pressure and heart rate without affecting the diastolic blood pressure. Thus, dependence on caffeine as a source of alertness among university students must be cautioned to avoid a sustained increase in ABP and accompanying complication.

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## Assessment of the Effect of Oral Dichlorvos Administration on Liver Function in Wistar Rats

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### Abstract

Dichlorvos is a synthetic organic chemical commonly used as an insecticide. Dichlorvos is widely used as an insecticide either solely or in a mixture with other insecticidal chemicals, primarily due to its affordability and ease of use and effectiveness. It is also used in agriculture as a pre-harvest or post-harvest pesticide. The work assesses the toxicity of orally administered dichlorvos in Wistar rats by assessing the activities of liver enzymes used as indices of liver function. Significantly higher ( $p < 0.05$ ) activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were observed in groups of animals exposed to dichlorvos. A dose-dependent manner of increase in the serum level of the enzymes. The findings of the study indicate that oral exposure to dichlorvos has possible toxicity to the liver.

**Keywords:** *Dichlorvos, Organophosphate, Liver, Toxicity*

### Introduction

Dichlorvos is a synthetic organic chemical commonly used as an insecticide. It is an organophosphate compound which is chemically 2,2-dichlorovinyl dimethyl phosphate, also commonly called DDVP. Dichlorvos, like other organophosphates, has been reported to be toxic to the extent of causing death in experimental animals. [1] It has also been implicated in some forms of cancer. [2, 3] Other reports have established no association between cumulative lifetime use of dichlorvos and risk of any cancer. [4]

Despite these facts, dichlorvos is widely used in developing countries as an insecticide in homes, offices, etc. and as an agricultural pesticide. [5] In Nigeria, dichlorvos is widely used as an insecticide either solely or in a mixture with other insecticidal chemicals, primarily due to its affordability and ease of use and effectiveness. It is also widely used in agriculture as a preharvest or postharvest pesticide. [6,7] Dichlorvos is the active compound in most locally prepared insecticides (commonly called *ota-piapia*) that are hawked on our streets, offices and homes. [8] In most cases, the individuals involved in the preparation, selling and using the insecticide are non-professionals, with no idea of the possible toxicological hazards posed by handling the

chemical. It is commonly understood that individuals and societies respond in diverse ways to hazardous substances. [9]

Dichlorvos enters the human system via various routes such as inhalation, oral and dermal. Organophosphate compounds generally possess high lipid solubility and are thus well absorbed from the skin, oral mucous membranes, conjunctiva, gastrointestinal and respiratory tracts. [10]

Dichlorvos is metabolised by the liver and excreted by the kidney. It has a relatively short biological half-life and is fairly rapidly metabolised and excreted. [11] The mechanism of action of dichlorvos is by acetylcholinesterase inhibition. Acetylcholinesterase is an enzyme responsible for the breakdown of acetylcholine, a neurotransmitter majorly used at the neuromuscular junction. The inhibition of acetylcholinesterase by dichlorvos results in the accumulation of acetylcholine in synapses negatively affecting nerve function. As an inhibitor, overdose of this organophosphorus compound leads to symptoms including weakness, headache, tightness in chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhoea, respiratory failure and abdominal cramps. [12]

Most studies on the toxicity of dichlorvos utilise routes other than oral in exposing experimental animals. With the way it is used locally by sprinkling around homes or to prolong the shelf life of agricultural food products, there is a high possibility of oral exposure to dichlorvos by ingesting contaminated food items or by direct ingestion. This work is aimed at ascertaining the toxicological effects of orally administered dichlorvos on the liver and kidneys of Wistar rats.

## Materials and Methods

### Preparation of Dichlorvos

Water was the medium used for administering dichlorvos to the laboratory animals via the oral route. As dichlorvos is hydrolysed by water.<sup>[13]</sup> the preparation is done a few minutes before administration.

### Treatment of Animals

Adult male Wistar rats weighting 150-200g were purchased from and kept in the animal house of the Department of Pharmacology, Bayero University/Aminu Kano Teaching Hospital, Kano. Throughout the study period, the animals had free access to normal diet and water *ad libitum*.

The animals were grouped into three based on the dose of dichlorvos/kg body weight into 1 mg/kg, 10 mg/kg and 20 mg/kg. A fourth group served as the control and received only the vehicle i.e. water. Each group comprised of five animals. The animals were given doses of the chemical once on daily basis by oral intubation. Standard procedures for animal handling were followed throughout.

### Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT),

The determination of the activities of the enzymes AST and ALT were carried out according to manufacturers instructions using Randox analytical kits. The analytical method was based on the method of Reitman and Frankel.<sup>[14]</sup>

### Alkaline Phosphatase (ALP)

ALP was analysed according to the Kind and King method.<sup>[15]</sup> In the method, when the serum is incubated with phenyl phosphate, the hydrolysis product, phenol, is condensed with 4-amino

antipyrine and then oxidized with alkaline ferricyanide to give a red complex that is measured at 500nm.

## Histological Analyses

Following humane sacrifice, the liver of the experimental animals was immediately dissected, fixed in 10% formalin<sup>[16]</sup> and taken to the Pathology Department of the Aminu Kano Teaching Hospital, Kano where the histological analyses were conducted with the assistance of a qualified histopathologist. The fixed tissues were dehydrated through ascending strengths of dehydrating fluid (alcohol), then cleaned in a mixture of absolute alcohol and xylene, then xylene alone to remove the alcohol. The tissues are then embedded in paraffin, cut into small pieces and stained using haematoxylin-eosin stain. The tissues were then observed under a light microscope at a magnification of x100.

## Results and Discussion

### Physical observation of experimental animals

The animals used in the studies were monitored for any change that can be assessed physically throughout the respective administration periods. The body weights of the animals were also monitored over the study period.

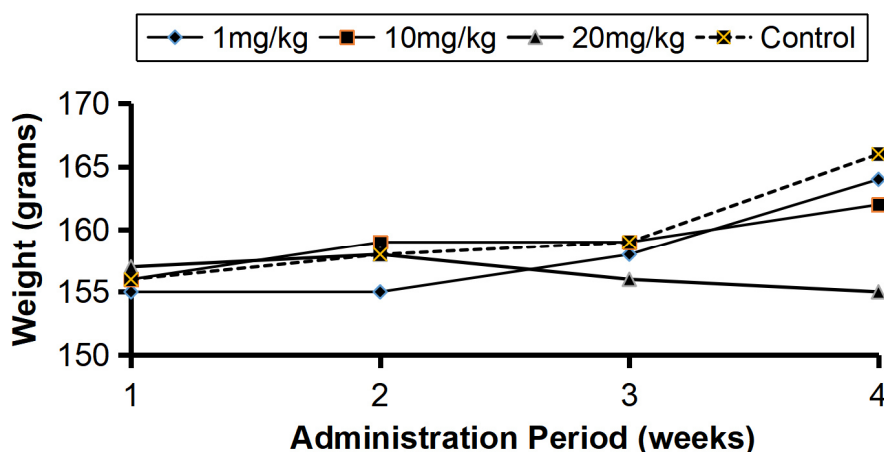
In all the animal groups administered 1mg/kg and 10mg/kg of dichlorvos for one week, there was no observed change or deviation in the normal animals' behaviour compared to the control group; animals in the 20mg/kg group were slightly less active than the other groups. In contrast, all the groups of animals administered dichlorvos for two and four weeks became increasingly sluggish and less active than the control groups. These observations were more prominent in the highest dose i.e. 20mg/kg group exposed for four weeks.

These observations can be linked to the effect of the chemical on acetyl cholinesterase and on the CNS. Dichlorvos reacts with and inactivates acetyl cholinesterase, the enzyme responsible for the degradation of the neurotransmitter acetyl choline.<sup>[17]</sup> This leads to the build-up in the concentration of acetyl choline resulting in general hyperexcitation of the CNS.<sup>[17]</sup> Convulsion and paralysis in skeletal

musculature are caused by brain anoxia as well as cholinergic effect within the CNS. [18] The less activity, pale and emaciated nature observed in especially the 20mg/kg group exposed for four weeks could be the result of the prolonged effect of the chemical on the liver, thus affecting the vital functions of the organ.

The general increase in mean weights of the Wistar rats in all treatment groups was observed up to two weeks of treatment. There were general decreases or slow increases in mean weights of the animals in all dichlorvos treated groups over the study period

(Figure 1). The observed lower rate of weight gain in the test animals can be linked to the effect of the chemical on the liver function. Obviously, any malfunction of the liver will significantly affect its basic metabolic functions which affect metabolic processes and homeostasis within the animals. Another reason for the slow increase in body weight in the Wistar rats could be due to the effect of dichlorvos in depleting glycogen stores. Dichlorvos has been reported to deplete glycogen stores by simultaneously stimulating glycogen phosphorylase and inhibition of glycogen synthesis. [19]



**Figure 1:** Mean weights of wistar rats exposed to oral dichlorvos for a period of 4 weeks

### Biochemical Analyses

As shown in Table 1, in animal groups exposed to dichlorvos for two weeks, there was a significantly higher ( $p < 0.05$ ) serum activities of the enzymes AST, ALT and ALP than the control in the 20mg/kg group. ALT activity in the 10mg/kg group was significantly higher ( $p < 0.05$ ) compared to the control. After four weeks of oral dichlorvos administration, the activities of all the enzymes analysed were higher in the test groups compared with the control. Significantly higher ( $p < 0.05$ ) activities were seen in AST, ALT, and ALP in the 20mg/kg group (Table 2).

Higher serum activities of AST and ALP are indicators of liver damage. ALT, a non-plasma specific enzyme is an indicator of liver damage because it has a high concentration in the liver of man, rat and dog. [20,21,22] ALP is present in many tissues, but that present in the normal serum is derived mainly from the liver cells, hence the

enzyme is a good marker for hepatic damage. [22, 23] AST, even though not liver-specific in most species, in the rat its concentration in other tissues is so low that it is considered liver-specific.

The observed increase in the activities of these enzymes are dose-dependent signifying that the toxic effect of the chemical is highly related to the dose of exposure; the highest activities of the enzymes were seen in the highest dose i.e. 20mg/kg group. Thus the activities of all the enzymes in the test groups increased from one week through to eight weeks administration period. This can be linked to the fact that dichlorvos taken orally is absorbed in the GIT and transported via the hepatic portal venous system into the liver. In the liver, the chemical is hydrolysed and thus does not reach the systemic circulation. [24] The hydrolysis products of dichlorvos are excreted in the urine. [25]

**Table 1:** Effect of Oral Administration of Dichlorvos for Two Weeks on AST, ALT and ALP in Wistar Rats

| GROUP   | AST<br>(u/l) | ALT<br>(u/l) | ALP<br>(u/100ml) |
|---------|--------------|--------------|------------------|
| 1mg/kg  | 10.75 ±0.30  | 10.00 ±0.78  | 4.50 ±0.46       |
| 10mg/kg | 11.20 ±0.10  | 11.15 ±0.18* | 5.00 ±0.14       |
| 20mg/kg | 11.94 ±0.24* | 11.50 ±0.47* | 5.33 ±0.18*      |
| Control | 11.00 ±0.30  | 10.05 ±0.45  | 4.35 ±0.42       |

Values are presented as mean ±standard deviation. Values bearing asterisk are significantly higher than the control in the same column

**Table 2:** Effect of Oral Administration of Dichlorvos for Four Weeks on AST, ALT, and ALP on Wistar Rats

| GROUP   | AST<br>(u/l) | ALT<br>(u/l) | ALP<br>(u/100ml) |
|---------|--------------|--------------|------------------|
| 1mg/kg  | 11.51 ±0.52  | 11.63 ±1.11  | 5.22 ±0.43       |
| 10mg/kg | 12.75 ±0.50  | 12.56 ±0.43  | 5.36 ±0.62       |
| 20mg/kg | 14.50 ±1.73* | 12.75 ±0.46* | 5.82 ±0.17*      |
| Control | 11.50 ±0.95  | 10.61 ±1.60  | 4.99 ±0.15       |

Values are presented as mean ±standard deviation

Values bearing asterisk are significantly higher than the control in the same column

### Histological Analyses

Histopathological analyses of the liver of the Wistar rats exposed to dichlorvos did not reveal any abnormality in the histological features in any test group compared to the control group. Even though the results of biochemical analyses were indicative of liver damage, the histopathological findings indicate that the damage done by dichlorvos at the dose and period exposed caused only minor damage to the liver.

### Conclusion

The present study found that oral dichlorvos has possible toxicity to the liver. Individuals involved in the use of the chemical should be at alert due to its possible toxic effects. This is especially important since the effects of the chemical were shown to be related to dose and time period of exposure.

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## Effect of Light at Night on Blood Level of Luteinizing Hormone, Follicle Stimulating Hormone and Testosterone in Male Wistar Rats

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### Abstract

Excessive exposure to artificial light or dark environments causes the disruption in the circadian rhythm of the day-night cycle which subsequently results in various diseases. The aim of this study was to investigate the effect of light at night on Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone in male Wistar rats. Thirty-five adult male Wistar rats (7-8 weeks old) mean weights of 110grams were randomly assigned into five groups of seven rats: Group A (control) was maintained under 12:12 light/dark cycle (12 hours light exposure). Group B was maintained under 18:6 light/dark cycle (18 hours light exposure). Group C was maintained under 6:18 light/dark cycle (6 hours light exposure). Group D was maintained under 0:24 light/dark cycle (24 hours dark exposure). Group E was maintained under 24:0 light/dark cycle (24 hours light exposure). The study last for three weeks. The rats were anaesthetized using chloroform by inhalation. The blood sample was collected by cardiac puncture. LH, FSH and testosterone were evaluated using ELISA kits according to the manufacturers' guidelines. The results showed that exposure to excessive artificial light or dark environments disrupted circadian rhythm. There were no statistically significant differences in weight gain among groups ( $p=0.805$ ). There were significant increase in the mean values of LH and Testosterone in rats exposed to 24 hours light compared to the control;  $64.45 \pm 1.59 \text{ mIU/ml}$  versus  $51.63 \pm 3.55 \text{ mIU/ml}$  ( $p=0.011$ ) and  $1.89 \pm 0.08 \text{ mIU/ml}$  versus  $1.28 \pm 0.06 \text{ mIU/ml}$  ( $p=0.001$ ) respectively. However, there was no significant difference in the levels of FSH between study and control groups ( $p=0.565$ ). This study demonstrates that exposure to excessive artificial light or dark environments disrupts circadian rhythm; exposure to artificial light at night significantly increases the concentration of LH and Testosterone but no effect on the concentration of FSH in male Wistar rats.

**Keywords:** *Circadian Rhythm, Luteinizing Hormone, Follicle Stimulating Hormone, Testosterone,*

### Introduction

One of the most rapid and radical changes that began at the end of the nineteenth century is the substitution of the natural 24 hours light/dark cycle for around-the-clock artificial light that differs markedly in the spectrum, intensity, and temporal patterning.<sup>[1]</sup> In mammals, environmental light is the main input that synchronizes the master circadian clock in the hypothalamic suprachiasmatic nucleus (SCN) to the outside world. The SCN communicates the external time of day to other brain areas, including areas that regulate sleep and food intake such as the ventrolateral preoptic nucleus and the lateral hypothalamus.<sup>[2]</sup> The SCN also communicates with peripheral clocks in metabolic tissues such as the liver and adipose tissue and regulates glucose metabolism.<sup>[3,4]</sup> Observational studies in humans shows that exposure to bedroom light at night was associated with insomnia, obesity, and dyslipidemia.<sup>[5]</sup> In nocturnal rodents, the bright light at night increased sleep and decreased food intake.<sup>[6]</sup> Long term exposure to constant light

disrupted circadian rhythmic behaviour, decreased total time spent awake and caused obesity and type 2 diabetes.<sup>[7]</sup> Circadian rhythm in human being and other mammals like rats can be assessed by measuring the circadian rhythmic profile in serum level of melatonin, cortisol, or core body temperature. In addition, rating scales such as the Morningness-Eveningness Questionnaire (MEQ) or Composite Scale also are useful to assess the sleep/wake cycle or circadian patterns of human.<sup>[8]</sup>

Light at Night (LAN), acting through endocrine disruption, have been implicated in an increased risk of metabolic diseases including breast cancer.<sup>[9]</sup> A series of studies have shown that light-dark patterns incident on the retina set the timing of the master clock.<sup>[10]</sup> Circadian disruption resulting from chronic exposure to irregular light-dark patterns plays a role in diseases such as cardiovascular disease, diabetes, obesity, and tumour growth.<sup>[11,12,13]</sup> One population at greater risk for exposure to LAN and irregular light-dark patterns

comprises shift workers working night shifts. Epidemiological studies show that this population is at increased risk for diseases, especially cancer.<sup>[14,15]</sup> The relationship between light exposure at night and obesity, metabolic disorders and cancer have been demonstrated in animal studies which show that nocturnal light exposure causes weight gain, hyperglycaemia and dyslipidaemia.<sup>[16,17]</sup> Several hormones were shown to have daily oscillations, and among these are melatonin, cortisol, gonadal steroids, prolactin, thyroid hormone and growth hormone.<sup>[18]</sup>

Infertility is one of the great challenges of modern healthcare.<sup>[19]</sup> Infertility affects about 8–12% of reproductive-aged couples worldwide.<sup>[20]</sup> Male factor is responsible for about 50% of cases of infertility overall: alone (~ 30%) or in association with a female factor of infertility (~ 20%).<sup>[20]</sup> Despite a complete diagnostic workup, in approximately 30% of cases of male infertility, no obvious cause for abnormal sperm parameters is found. This occurrence is defined as “idiopathic infertility”.<sup>[21]</sup> Many previous studies have reported that compared to normal light cycle, both increasing the light and decreasing the light exposure can affect ovarian follicular development, an increase in androgen and deregulation of sexual cycles.<sup>[22,23]</sup> These investigations suggest that photoperiod affect the reproductive system and the secretion of female sex hormones. There is a paucity of data on the effect of light exposure at night on male reproductive system using experimental animals. This study was designed to investigate the effect of light at night on plasma concentration on LH, FSH and testosterone in adult male Wistar rats.

## Methods

### Experimental Design

The study comprised of thirty-five adult male Wistar rats, 7-8 weeks old, mean weights of 110grams. The rats were sourced from the Anatomy Department and Biological Science Department, Bayero University Kano, Nigeria. Prior to the commencement of the experiment, all animals were maintained in a 12:12 LD cycle for four weeks to ensure that the animals entrained to the light cycle according to Farhadi *et al.*<sup>[24]</sup> The rats were randomly assigned into five different groups, seven rats in each group as follows:

Group A (control) maintained under 12:12 light/dark cycle (12 hours light exposure).

Group B maintained under 18:6 light/dark cycle (18 hours light exposure).

Group C maintained under 6:18 light/dark cycle (6 hours light exposure).

Group D maintained under 0: 24 light/dark cycle (24 hours dark exposure).

Group E maintained under 24:0 light/dark cycle (24 hours light exposure).

The rechargeable LED Emergency Lamp (Lonen, China) was assessed in Physics Department, Bayero University, Kano, Nigeria for separation of its colour components using an advanced spectrometer (CK030, India) and it was found to contain all components of white light (ROYGBIV). The light intensity used was 316.5 lux which is within the range of 200 to 500 lux.<sup>[16]</sup> The study lasted for three weeks.

### Measuring of Core Body Temperature

Two days prior to termination of the study, the core body temperature of the animals was determined at three hours interval for 24 hours using a digital thermometer (Bokang, China) through rectal route (about 1.5cm of thermometer inserted into the rectum). The core body temperature was measured to assess the circadian rhythm in the study and control groups so as to determine whether there is a disruption of circadian rhythm in the study groups as a result of interventions compared to control.

### Samples Collection

At the end of three weeks, the rats were anaesthetized using chloroform by inhalation. The blood sample was collected by a cardiac puncture; 4ml of the blood sample was collected from each rat into EDTA container and centrifuged at 3000 rpm for 30 minutes (relative centrifugal force=504g). The plasma was collected in a plain container and stored at frozen temperature (-20°C).<sup>[25]</sup> The plasma was used for LH, FSH and testosterone analysis.

### Determination of LH

Plasma concentration of LH was determined by Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available LH ELISA kit (CALBIOTECH, USA). The LH ELISA is a solid phase assay using streptavidin/biotin method. The samples and anti-LH/anti-Biotin conjugate were added to the wells coated with streptavidin. LH in the plasma forms a sandwich between specific antibodies labelled with biotin and horseradish peroxidase (HRP). 25µL of LH standards, control and plasma were added into assigned wells followed by addition of 100µL of enzyme conjugate into each well. The plate was covered and incubated for 60minutes at room temperature (27°C). The liquid was removed from all wells and washed three times with 300µL of wash buffer. 100µL of

tetramethylbenzidine (TMB) substrate was added to all wells and then incubated for 15 minutes at room temperature. 50µL of stop solution was then added to all the wells and rocked gently for 15 seconds. The absorbance was read at 450nm after 15 minutes using microplate reader spectrophotometer (LT-1200, USA). The concentration of LH in the sample was calculated from the calibration curve of the concentration of standards and their absorbance.

#### Determination of FSH

Plasma concentration of FSH was determined by ELISA using a commercially available ELISA kit (CALBIOTECH, USA). The FSH-ELISA is a solid phase assay using streptavidin/biotin method. The samples and anti-FSH/anti-Biotin conjugate were added to the wells coated with streptavidin. FSH in the plasma forms a sandwich between specific antibodies labelled with biotin and HRP. 50µL of FSH standards, control and plasma were added into assigned wells followed by addition of 100µL of enzyme conjugate into each well. The plate was covered and incubated for 60 minutes at room temperature. The liquid was removed from all wells and washed three times with 300µL of wash buffer. 100µL of TMB substrate was added to all the wells and then incubated for 15 minutes at room temperature (27°C). 50µL of stop solution was then added to all wells and rocked gently for 15 seconds. The absorbance was read at 450nm after 15 minutes using microplate reader spectrophotometer (LT-1200, USA). The concentration of FSH in the sample was calculated from the calibration curve of the concentration of standards and their absorbance.

#### Determination of Testosterone

Plasma concentration of testosterone was determined by ELISA using a commercially available ELISA kit

(CALBIOTECH, USA). The testosterone test kit is a solid phase competitive ELISA. The samples, working Testosterone-HRP Conjugate and anti-Testosterone-biotin solution are added to the wells coated with streptavidin. Testosterone in the plasma competes with the Testosterone enzyme (HRP) conjugate for binding sites. 50µL of testosterone standards, control and plasma were added into assigned wells. 100µL of working testosterone-enzyme conjugate reagent was added into each well followed by 50µL biotin reagent and mixed gently for 20 seconds. The plate was covered and incubated for 60 minutes at room temperature. The liquid was removed from all wells and washed three times with 300µL of wash buffer. 100µL of TMB substrate was added to all wells and then incubated for 30 minutes at room temperature (27°C). 50µL of stop solution was then added to all wells and rocked gently for 20 seconds. The absorbance was read at 450nm after 15 minutes using microplate reader spectrophotometer (LT-1200, USA). The concentration of testosterone in samples was calculated from the calibration curve of the concentration of standards.

#### Statistical Analysis

The data collected were analyzed using IBM SPSS statistical package version 23.0. Variables were tested for normality using Shapiro-Wilk test and Z values of kurtosis and skewness. Results were presented as mean  $\pm$  standard error of the mean. 24 hours core body temperature circadian rhythm was analyzed using Cosinor Software (COSINOR 2.4 release). Inferential Statistics of analysis of variance (ANOVA) with *post hoc* test of Bonferroni was computed for the determination of statistically significant differences. P-values of <0.05 was considered statistically significant.

### Results

**Table 1: Circadian Rhythm of Core Body Temperature (in Degree Celsius) of Rats Within 24 Hours**

| GROUPS<br>(n=7)      | MEAN $\pm$ SEM OF TEMPERATURE IN (°C) AT VARIOUS TIME INTERVALS |                |                 |                 |                 |                |                |                 | F-value | p-value |
|----------------------|-----------------------------------------------------------------|----------------|-----------------|-----------------|-----------------|----------------|----------------|-----------------|---------|---------|
|                      | 10hr                                                            | 13hr           | 16hr            | 19hr            | 22hr            | 1hr            | 4hr            | 7hr             |         |         |
| GROUP A<br>(CONTROL) | 37.8 $\pm$ 0.2                                                  | 37.1 $\pm$ 0.9 | 38.4 $\pm$ 0.1  | 38.6 $\pm$ 0.1* | 38.0 $\pm$ 0.1  | 37.5 $\pm$ 0.1 | 37.1 $\pm$ 0.1 | 36.5 $\pm$ 0.1* | 24.136  | 0.001*  |
| GROUP B              | 37.8 $\pm$ 0.1                                                  | 37.0 $\pm$ 0.1 | 36.9 $\pm$ 0.2  | 37.5 $\pm$ 0.5  | 37.6 $\pm$ 0.1* | 37.3 $\pm$ 0.1 | 37.1 $\pm$ 0.2 | 36.5 $\pm$ 0.1* | 4.004   | 0.002*  |
| GROUP C              | 38.0 $\pm$ 0.1                                                  | 37.2 $\pm$ 0.1 | 37.3 $\pm$ 0.1  | 37.5 $\pm$ 0.2  | 38.1 $\pm$ 0.1* | 37.6 $\pm$ 0.1 | 37.1 $\pm$ 0.2 | 36.9 $\pm$ 0.1* | 13.374  | 0.001*  |
| GROUP D              | 37.4 $\pm$ 0.1                                                  | 37.3 $\pm$ 0.2 | 37.3 $\pm$ 0.1* | 38.0 $\pm$ 0.2  | 38.0 $\pm$ 0.1* | 37.5 $\pm$ 0.1 | 37.1 $\pm$ 0.1 | 37.0 $\pm$ 0.2  | 9.083   | 0.001*  |
| GROUP E              | 38.2 $\pm$ 0.1                                                  | 37.5 $\pm$ 0.1 | 37.7 $\pm$ 0.1* | 37.6 $\pm$ 0.1  | 37.6 $\pm$ 0.1  | 37.4 $\pm$ 0.1 | 37.1 $\pm$ 0.2 | 36.9 $\pm$ 0.1* | 12.566  | 0.001*  |

n= number of rat per group, L/D= light-dark cycle, SEM= standard error of mean, °C= degree Celsius, hr=hour,

\*P<0.05 means Significant values. Values with superscript indicated where there was a significant difference.

Table 1 shows the circadian rhythm of core body temperature (in degree Celsius) of rats within 24 hours. Repeated measures ANOVA with *posthoc*

Bonferroni comparison indicated that there was a significant difference ( $p < 0.05$ ) in both control and experimental groups at different time intervals.

**Table 2:** Cosinor analysis of 24 hours temperature rhythm of the rats

| GROUPS (n=7)      | MESOR  | AMPLITUDE | ACROPHASE       |
|-------------------|--------|-----------|-----------------|
| GROUP A (CONTROL) | 37.6°C | 0.7       | -82° (18:30hr)  |
| GROUP B           | 37.2°C | 0.2       | -61° (19:55hr)  |
| GROUP C           | 37.4°C | 0.2       | -44° (21:01hr)  |
| GROUP D           | 37.4°C | 0.3       | -56° (20:13hr)  |
| GROUP E           | 37.5°C | 0.2       | +234° (15:37hr) |

MESOR= Midline estimating statistic of rhythm, n= number of rats per group, L/D= light-dark cycle, °C= degree Celsius, hr = hour.

The peak (acrophase) in group A (control), B, C, D and E were 18:30hour, 19:55hour, 21:01hour, 20:13hour and 15:37hour respectively. Midline estimating statistic of rhythm (Mesor), the value halfway between the highest and lowest values of the cosine curve were 37.6°C, 37.2°C, 37.4°C, 37.4°C and 37.5°C in group A (control), B, C, D and E respectively. Amplitude, the distances from the mesor value to the Peak were 0.7, 0.2, 0.2, 0.3 and 0.2 in group A (control), B, C, D and E respectively (table 2)

**Table 3:** Effect of light exposure on CBT, WTG, LH, FSH and testosterone in male wister rats

|              | Group A<br>(12:12 L/D, control) | Group B<br>(18:6 L/D) | Group C<br>(6:18 L/D) | Group D<br>(0:24 L/D) | Group E<br>(24:0 L/D) |         |         |
|--------------|---------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|---------|---------|
| Variable     | n (7)                           | n (7)                 | n (7)                 | n (7)                 | n (7)                 | F-Value | P-value |
| CBT (°C)     | 37.78±0.22                      | 37.30±0.19            | 37.31±0.18            | 37.51±0.16            | 37.45±0.15            | 1.132   | 0.360   |
| WTG (g)      | 43.42±7.40                      | 48.00±13.17           | 60.57±12.84           | 52.28±12.09           | 43.42±10.04           | 0.403   | 0.805   |
| LH (mIU/ml)  | 51.63±3.55                      | 62.31±1.55            | 61.00±3.05            | 60.97±2.0             | 64.45±1.59*           | 3.899   | 0.011*  |
| FSH (mIU/ml) | 48.37±0.37                      | 47.05±1.57            | 48.55±0.44            | 48.52±0.23            | 48.61±0.22            | 0.752   | 0.565   |
| TST (mIU/ml) | 1.28±0.06                       | 1.68±0.08             | 1.37±0.08             | 1.77±0.07             | 1.89±0.08*            | 11.241  | 0.001*  |

n= number of rats per group, L/D=light dark cycle, CBT= Core Body Temperature, WTG=Weight Gain, LH=Luteinizing Hormone, FSH=Follicle Stimulation Hormone, TST=Testosterone. \* =Significant ( $p < 0.05$ ) values relative to control

Table 3 above shows that there was no significant difference in mean weight gain and core body temperature among the control and experimental groups ( $p > 0.05$ ). There was a significant increase in the mean level of testosterone ( $p = 0.001$ ) and LH ( $p = 0.011$ ) in 24 hours light exposure group (group E) as compared to control (Table 3). There was no significant difference in FSH across the groups ( $p = 0.565$ ) (table 3).

### Discussion

The present study determined the circadian rhythm of male Wistar rats in experimental and control groups by measuring 24 hours core body temperature through the rectal route. It also assessed the disruption of circadian rhythm by exposure of artificial light at night and its subsequent effect on LH, FSH and testosterone. Cosinor analysis of 24 hours core body

temperature rhythm revealed that the time at which the temperature reaches the peak (acrophase) is 18:30 hour in group A (control). This is closely similar to the study conducted by Garance *et al.* [26] who found that the core body temperature acrophase of rats under *ad libitum*, 12:12 L/D and activity free conditions were 18:40 hour.

There was phase shift, displacement of a curve along the time axis in group B (19:55 hour), group C (21:01 hour), group D (20:13 hour) and group E (15:37 hour) compared to group A (control) (18:30 hour) indicating disruption of circadian rhythm in experimental groups (group B, C, D and E). This is in line with the study by Satoh *et al.* [27] who found that there was phase shift of core body temperature in male mice after an abrupt change in environmental light phases. It is also in line

with the studies of Stevens and Rea, [28] Fu and Lee [29] and Vinogradova and Anisimov[30] who found that artificial light at night using electronic devices disrupt circadian rhythm which subsequently leads to cancer, cardiovascular and metabolic diseases.

There was no significant difference in weight gain among the control and experimental groups. The weight gain of group E (24 hours light) and control groups were similar. This is probably due to a similar feeding habit and activity level of the rats in the two different groups. These findings is similar to the study conducted by Wideman and Murphy [31] who found that rats maintained under constant light condition do not appear to gain weight compared to rats exposed to standard light/dark cycle, contrary to the findings of Fonkenet *al.*[32] who established a positive relationship between nocturnal light exposure and weight gain in animal studies. It is also contrary to the study by Verma *et al.* [33] who stated that “continuous light-exposed rats show a significant increase in the body weight and non-significant changes in the bodyweight of continuous dark exposed group”.

The present study also investigated the effect of circadian rhythms disruption on male reproductive hormones. This study showed that there was a significant increase in the mean level of testosterone ( $p=0.001$ ) and LH ( $p=0.011$ ) in 24 hours light exposure group (group E) as compared to control. This is similar to a previous study conducted by Kang *et al.*[34] which found that serum testosterone levels of female rats were significantly increased in a continuous light environment.[21] There was no significant difference in FSH across the groups ( $p=0.565$ ). High level of testosterone predisposes male rats to prostate enlargement [35] and polycystic ovary syndrome (PCOS) in female rats with subsequent infertility.[34] The significant increase in the mean level of testosterone and LH in 24 hours light exposure group is probably due to absence of inhibitory effect of melatonin on LH and testosterone secretion[36] which resulted from the light inhibitory effect on melatonin secretion.

## Conclusion

The results of this study have shown that exposure to excessive artificial light or dark environments disrupts circadian rhythm which significantly increases the level of luteinizing and testosterone hormones but does not affect follicle-stimulating hormone in adult male Wister rats.

## Conflict of Interest

The authors have declared that there is no conflict of interest.

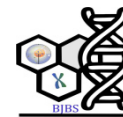
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## Evaluation of Hepatoprotective effect of the Aqueous Extract of *Azadirachta indica* (Neem) Stem Bark against Paracetamol Induced Liver Damage in Adult Wistar Rats

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### Abstract

*Azadirachta indica* (neem) is a widespread and well known medicinal plant that has been found to contain certain compounds that have been found to be important in anti-oxidation and hence hepatoprotection. In this study, the effect of oral administration of the aqueous stem bark extract of Neem was evaluated on paracetamol induced hepatotoxicity in Wistar Rats. The study lasted for one week and twenty-five Wistar Rats were divided into five groups of five rats each according to their weight ranges. Group one was fed normally with vital feed and water throughout the experimental period. Group two was given (2000mg/kg) paracetamol on the 7<sup>th</sup> day. Groups three was given low dose of the extract (500mg/kg for 6 days and 2000mg/kg paracetamol on the 7<sup>th</sup> day. Group four was given high dose of the extract (1000mg/kg) for 6 days and 100mg/kg. While Group five was given 100mg/kg of Silymarin for 6 days and 2000mg/kg of paracetamol on the 7<sup>th</sup> day. The rats were weighed and humanely sacrificed on the eight day that is twelve hours after the last treatment. Incision was made to expose the abdominal viscera where the liver and the kidney were fixed in neutral buffered formalin and the tissues were processed histologically using normal routine method, sectioned and stained with Hematoxylin and Eosin and slides were observed histologically. Paracetamol (2000 mg/kg) significantly ( $P<0.001$ ) elevated the levels of AST, ALT and ALP when compared with control. The extent of liver damage was evidenced by elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and by histopathological observations of liver sections showed severe blood congestion in central vein, infiltration of inflammatory cells, irregular hepatocyte arrangement, focal necrosis and sinusoidal dilatation. Oral administration of low dose of aqueous *A. indica* stem bark extract (500 mg/kg), high dose of extract (1000mg/kg), the extract significantly reduced these elevated levels of AST, ALT and ALP statistically when compared with the paracetamol treated group. It was then concluded that oral administration of aqueous extract of *A. indica* stem bark has hepatoprotective effect against paracetamol induced liver damage.

**Keywords:** Hepatoprotective, Aqueous extract. *Azadirachta indica*, Paracetamol, Liver, Wistar rats

### Introduction

The liver is a greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it manufactures bile which aid in fat digestion and neutralizes and remove toxins through the bowels.<sup>[1]</sup> The liver is a vital organ of paramount importance involved in the management of metabolic function and detoxification from exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism.<sup>[2]</sup> The ability of the liver to

perform its functions is often compromised by numerous substances we are exposed to on a daily basis; these substances include certain medicinal agents which when taken in over doses and sometimes when introduced within therapeutic range injures the organ.<sup>[1]</sup> Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in tissue reduced glutathione (GSH) levels. In addition, serum level of many biochemical markers like: triglycerides, cholesterol, bilirubin, alkaline phosphatase, Serum

glutamate oxaloacetate (SGOT) and Serum glutamate pyruvate transaminase (SGPT) are elevated.<sup>[3]</sup>

Paracetamol is a drug which has analgesic and antipyretic effects. Its therapeutic effect is similar to salicylates, but it lacks anti-inflammatory, antiplatelet and gastric ulcerative effects.<sup>[4]</sup> It belongs to the group of anilides which are organic heterocyclic compounds derived from oxoacids. It is a widely used drug used for the treatment of headaches, fever, and other minor aches or pains.<sup>[4]</sup>

*Azadirachta indica* (neem) is a tropical evergreen tree native to Indian sub-continent. Most of the plant parts such as fruits, seeds, leaves, bark and roots contain compounds with proven antiviral, antiseptic, antipyretic, anti-inflammatory, antiulcer and antifungal uses.<sup>[5,6,7,8]</sup> It has great potential in the fields of pest management, environment protection and medicine. Neem is a natural source of eco-friendly insecticides, pesticides and agrochemicals.<sup>[9]</sup>

Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles.<sup>[10,11]</sup> The active principles are slightly hydrophilic, but freely lipophilic and highly soluble in organic solvents like, hydrocarbon, alcohols, ketones and esters.<sup>[12]</sup>

Neem stem bark is seen to be rich in certain substances which could be beneficial in the treatment of liver damage and liver disease,<sup>[13]</sup> hence the need for this study to evaluate the hepatoprotective effect of aqueous extract of neem stem bark so as to find a much more suitable, relatively safer and cheaper alternative to the

conventional drug for treatment of liver damage there by making the study significance.

## Materials And Methods

### Preparation of Extract

The extraction of the neem stem bark was done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The cold maceration method of extraction was used for the preparation of the aqueous extract. Different concentrations were measured using an electronic weighing balance and dissolved in 20mls of distilled water. This make up the working solutions. From freshly prepared working solution, little portion was measured out for animal administration. Different doses of extract were administered to experimental animals by oral gavages using oro-gastric tubes for experimental period.

### Acute Toxicity Study of Neem

The LD50 experiment was done in the Animal House, Department of Human Anatomy, Ahmadu Bello University, Zaria. Oral acute toxicity study was performed to evaluate the acute toxic effects and to determine minimum lethal dose (LD50) of the plant extract. A total number of twelve (12) apparently healthy wistar rats (male and female) weighing 100-130g were used for the experiment. The up and down method of determining LD50 was used. The aqueous extract was administered orally to the different groups (n=6) of overnight fasted rats for 12 hours in two stages. In the up phase of determining the LD50, six rats with an average weight between 100-110g were randomly placed into three groups of two rats each and they were treated with different concentrations of the extract as seen in the table below.

**Table 1:** Up Phase of Determining LD50.

| GROUPS | Administrations                  |
|--------|----------------------------------|
| 1      | 1000mg/kg body weight of extract |
| 2      | 2000mg/kg body weight of extract |
| 3      | 3000mg/kg body weight of extract |

Before the administration, rats were fasted over night for 12 hours. After the administration, they were observed after 24, 48 and 72 hours for any morphological change, signs of toxicity and adverse effect and mortality and weighed daily for three days. In the down phase of determining LD50, the

procedure was repeated as in the up phase using three rats with an average weight of 102g which were randomly placed in three groups of one rat each and were treated with different concentrations of the extract as seen in the table below.

**Table 2:** Down Phase of Determining LD50.

| Groups | Administrations                  |
|--------|----------------------------------|
| 1      | 1000mg/kg body weight of extract |
| 2      | 1500mg/kg body weight of extract |
| 3      | 2500mg/kg body weight of extract |

### Experimental Design

**Table 3:** Experimental Design of the Research (animal grouping and treatment).

| Groups      | Administrations                                                                          |
|-------------|------------------------------------------------------------------------------------------|
| 1 (Control) | Distilled water only                                                                     |
| 2           | 2000mg/kg of Paracetamol on 7 <sup>th</sup> day                                          |
| 3           | Low dose of extract (500mg/kg) for 6 days + Paracetamol 2000mg/kg on 7 <sup>th</sup> day |
| 4           | High dose of extract(1000mg/kg)for 6 days + Paracetamol on 7 <sup>th</sup> day           |
| 5           | Silymarin (100 mg/kg) for 6 days + Paracetamol (2000mg/kg) on 7 <sup>th</sup> day        |

### Histological analysis

After fixing in 10% formal saline, liver tissue were dehydrated with ethanol solution at increasing graded concentrations of 70-75-80-85-95-100% absolute ethanol, then it was cleared using xylene. The tissue was then embedded in paraffin wax 62°C, then sectioned at 5 µm and stained using hematoxylin and eosin staining and light microscopy was used to observe the tissues.

### Biochemical Analysis

A section of the liver was homogenized using physiological buffered solution and transferred into ethylenediaminetetraacetic acid (EDTA) bottles then centrifuged for five minutes at 2500 revolutions per minute. The supernatant was drawn using an aspirator and transferred into labelled plane sample bottles. The supernatant were used for biochemical analysis to determine the activities of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) using reagent kits (Quinima Clinica Applicada, S.A. Spain) and according to the manufacturer's instructions.

### Statistical Analysis

Results were analysed using statistical software. SPSS version 21.0. Results were expressed as mean ± Standard Error of Mean. Results were further analysed using paired T test and one way ANOVA with least significance difference(LSD) post hoc test; values were considered significant when  $p < 0.05$ .

The percentage body weights were calculated using organ/body weight ratio.

### Results

#### Acute Toxicity

The histology of the liver showed little to moderate damage in the treatment groups from 1000mg/kg to 3000mg/kg of the extracts. It was after the acute toxicity test that the doses to be used for the experiments were chosen to be 500 and 1000mg/kg of extracts.

#### Biochemical

Group one which serves as the control group that received only distilled water showed normal levels of liver enzymes (ALP, AST and ALT) which depicts that liver damage has not occurred. Group two that received paracetamol only on the seventh day showed elevation of the three liver enzymes (ALP, AST, ALT) beyond the normal range when compared to the control and the difference is statistically significant. The low dose of the extract treated group has lower levels in liver enzymes ALT and ALP and a little higher level of AST when compared to silymarin. Liver toxicity can be assessed by checking of its serum enzymes level. Examples of enzymes like Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Bilirubin increase in the level of these enzymes will indicate hepatotoxicity.

**Table 4:** Parameters of Liver Function in Control and Experimental Animals

|           | % Body Weight | AST (U/L)       | ALT (U/L)       | ALP (U/L)        |
|-----------|---------------|-----------------|-----------------|------------------|
| Control   | 5.06 ± 0.65   | 31.00 ± 4.00    | 46.50 ± 1.50    | 102.00 ± 11.00   |
| PCM       | 5.42 ± 0.01   | 67.00 ± 1.00*** | 82.00 ± 1.00*** | 170.00 ± 1.00*** |
| LD EX+PCM | 6.12 ± 0.33   | 40.25 ± 1.38*** | 52.50 ± 1.85    | 121.50 ± 1.55**  |
| HD EX+PCM | 6.25 ± 0.54   | 45.40 ± 1.91*** | 60.60 ± 1.86**  | 131.00 ± 2.76*** |
| SIL       | 5.06 ± 0.42   | 41.80 ± 1.39**  | 51.60 ± 2.77    | 122.60 ± 2.42**  |

MEAN±SEM, One way ANOVA with LSD post hoc test, \*= $p < 0.05$ , \*\*= $p < 0.005$ , \*\*\*= $p < 0.001$  significance difference when compared to control; CTL(Control), PCM(Paracetamol), LD EX+PCM (Low Dose(500mg/kg) of extract and Paracetamol), HD EX+PCM (High Dose (1000mg/kg) of extract and Paracetamol), SIM (Silymarin). AST (Aspartate amino transferase), ALT (Alanine amino transferase), ALP (Alkaline phosphatase).

### Histopathological

Histopathological observation of tissues sections of the liver of the experimental animals (wistar rats) revealed the following:

The liver sections of animals in control group (group 1) showed normal cyto-architecture of liver parenchyma; the characteristics of hepatic lobule unit: centrilobular venules (central vein) and an array of regular arrangement of liver cells (hepatocytes) radiating from the central vein separated by vascular spaces called the hepatic sinusoids, kupffer cells within the sinusoids are also visible (plate 1). The liver sections in rats treated with paracetamol (2000mg/kg) revealed severe histopathological distortion of liver parenchyma which manifested as severe blood congestion in central vein, infiltration by inflammatory cells, irregular hepatocyte arrangement, focal necrosis and sinusoidal dilatation, presence of vacuolations (plate 2).

The liver sections of rats in group 3 administered low dose of neem extract (500mg/kg) aqueous stem bark extract of neem followed by administration of paracetamol (2000mg/kg) revealed mild liver damage, central vein more intact than in paracetamol

treated groups, mild scattering of hepatocytes, milder enlargement of hepatocytes and presence of vacuolations not like in the paracetamol treated group that is severe showing there is tissue repair that has occurred in the liver tissue (plate 3).

The liver section of the rats in group 4 administered high dose of extract (1000mg/kg) aqueous stem bark extract of neem followed by administration of paracetamol (2000mg/kg) revealed distorted histoarchitecture, inflamed hepatocytes due to tissue damage, sinusoidal dilatation and focal necrosis, blood congestion in central vein, presence of vacuolations and central vein enlargement but the distortion not severe like in paracetamol group but more severe than that of the low dose of extract group (plate 4).

The liver sections of rats in group 5 treated with Silymarin (100mg/kg) and paracetamol (2000mg/kg) showed mild histoarchitectural distortion of liver parenchyma which manifest as mild sinusoidal dilatation and blood congestion in central vein (plate 5).



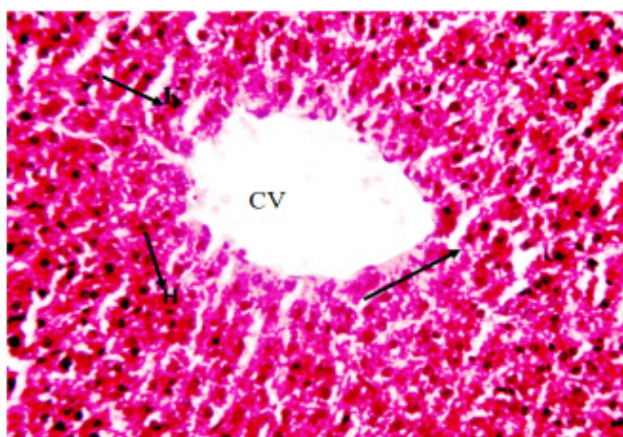


Plate 1: Section of the liver of the control group 1 administered distilled water only showing CV (central vein), SN (sinusoids), H (hepatocytes), K(Kupffer cells), (H&E,  $\times 250$ ).

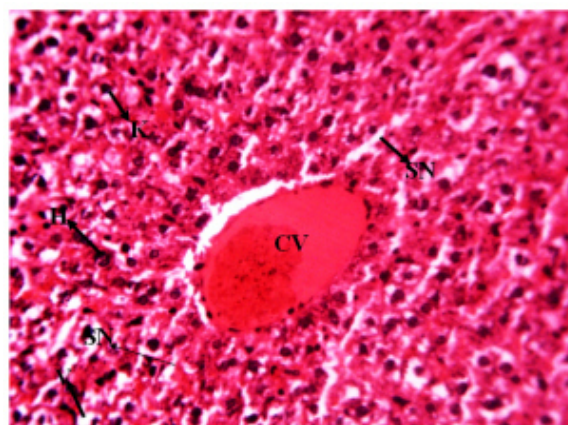


Plate 2: Section of the liver of the experimental group 2 administered 2000mg/kg paracetamol CV (central vein), SN (sinusoidal dilatation), H (hepatocytes), K (Kupffer cells), V (Vacoulations), (H&E,  $\times 250$ )

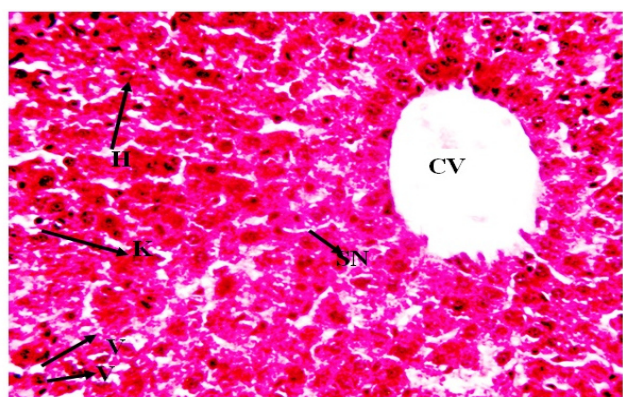


Plate3: Section of the liver of experimental group 3 administered 2000mg/kg paracetamol + 500 mg/kg *A. indica* aqueous stem bark extract CV (central vein), SN (sinusoids), H (hepatocytes), K (Kupffer cells), V (Vacoulations) (H&E, X250).

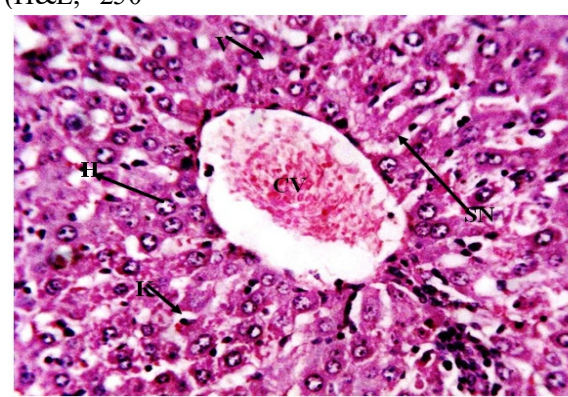


Plate4: Section of the liver of experimental group 4 administered 2000 mg/kg paracetamol +1000 mg/kg *A. indica* aqueous stem bark extract CV (central vein), SN (sinusoids), H(hepatocytes), K (Kupffer cells), V (Vacoulations) (H&E, X250).

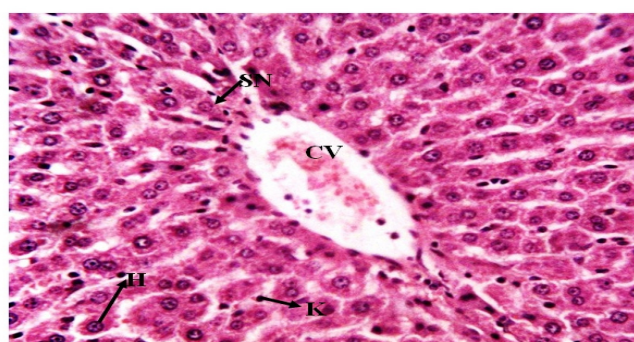


Plate5: Section of the liver of experimental group 5 administered 2000 mg/kg paracetamol +100 mg/kg of Silymarin( H&E, X250). CV (central vein), SN (sinusoids), H (hepatocytes), K(Kupffer cells) (H&E, X250).

## Discussion

The primary function of the liver is to maintain body homeostasis, besides it plays a key role in metabolism, detoxification, and inflammatory response [14]. The liver section of group one rats revealed normal histoarchitecture of hepatic lobule unit (central veins, portal triad, hepatocytes, kupffer cells and sinusoids) and also normal cytoarchitecture of the liver tissue.

Paracetamol has been used as a successful experimental model to evaluate the efficacy of hepatoprotective agents. [15] Due to liver injury caused by paracetamol overdose, the transport function of the hepatocytes gets disturbed resulting in the leakage of the plasma membrane [16] thus causing an increase in serum enzyme levels. In the group that was given paracetamol overdose, the histology of the liver of the rats in the group showed severe blood congestion in central vein, infiltration of inflammatory cells, irregular hepatocyte arrangement, focal necrosis and sinusoidal dilatation showing the hepatotoxic effect of paracetamol which is in line with the biochemical assay of the liver enzymes in which all the three liver enzymes are elevated well above the levels of the other groups.

Neem is said to possess hepatoprotective effect against many hepatotoxic agents such as paracetamol. [17] This work was aimed at using different dosage of the aqueous extract of neem stem bark to see if it have a hepatoprotective effect as reported by Gomase *et al.* [18] but using the extract of the stem bark using carbontetrachloride as the hepatotoxin.

The histology of the group that was administered low dose shows a better preservation of the liver cytoarchitecture than the high dose of the extract and even silymarin. The distortion of the cytoarchitecture of the liver tissue was not as bad as that as the group that received paracetamol only. This shows that the hepatoprotective effect of the extract or its effect against hepatotoxicity reduces as the dosage is increased. At lower dosage, it has more hepatoprotective and therapeutic effect than at higher dose. The liver sections of rats in group 5 showed mild histoarchitectural distortion of liver parenchyma.

Histopathology of the liver showed that the aqueous extract of *A.indica* stem bark possesses a hepatoprotective effect. Due to the fact that milder distortion is seen in cytoarchitecture of the liver tissue in the low dose of neem stem bark extract group than in the higher dosage group, this shows neem stem bark aqueous extract at low dose has more hepatoprotective, therapeutic and beneficial effect on the liver than the higher dose. The ability of silymarin in preventing drug induce hepatotoxicity is associated with its ability to act as a radical scavenger, thereby protecting membrane permeability. [19] Silymarin is used as a reference drug to compare hepatoprotective effects of other plants due to its proven efficacy in hepatoprotection. In the group that were administered 100mg/kg of silymarin for the first six days and 2000mg/kg of paracetamol on the seventh day, the histoarchitecture of the liver tissue was normal with only mild distortion, sinusoidal dilatation which shows it reduces the damage that was caused by paracetamol on the liver therefore hepatoprotective.

This present study focused on the magnitude of recovery of paracetamol injured hepatocytes by different doses of the aqueous extract of *A. indica* stem bark using silymarin as a standard hepatoprotective reference drug. This shows that oral administration of the two different doses (500mg/kg and 1000mg/kg) of the aqueous extract of stem bark of *Azadirachta indica* on paracetamol induced liver damage resulted in gradual normalization of the activities of AST, ALP, ALT and also histologically by restoring the normal histoarchitecture of the liver tissue, therefore a strong evidence confirming hepatoprotective effects of the extracts studied.

## Conclusion

Aqueous stem bark extract of *Azadirachta indica* (neem) has potential hepatoprotection against paracetamol induced liver damage or hepatotoxicity by showing signs of liver restoration and decrease in the serum levels of liver enzymes, the low dose of these extract is more beneficial on the liver than the high dose and to a lesser extent the standard hepatoprotective drug (silymarin).

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## Finger Millet Supplementation Alleviate Urea and Serum Electrolytes in Mercury Chloride Induced Nephrotoxicity in Wister Rats.

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### Abstract

Whole-grain consumption is being regarded as a safe approach for toxicity. Millets are also whole grains and their consumption has been shown to be inversely associated with metabolic diseases. Finger millet (*Eleusine coracana*) is an important food crop among Africans and Indians. It is regarded as “wonder grain/super cereal” due to health-promoting effects. Heavy metals such as mercury, when exposed to, pose great health problems, when absorbed, inhaled or ingested into the bloodstream, they damage tissues like the kidney, brain and liver. Urea, creatinine, and serum electrolytes (sodium, Na<sup>+</sup>, potassium, K<sup>+</sup>, chloride, Cl<sup>-</sup> and bicarbonate, HCO<sub>3</sub><sup>-</sup>) are very important parameters for maintaining the body’s fluid balance. Their imbalance results in several disorders such as dehydration. This study was designed to investigate the effect of finger millet on urea and serum electrolytes in mercury chloride (HgCl<sub>2</sub>) induced toxicity in wistar rats. Toxicity was chemically induced by mercury chloride through oral route of administration on alternate days for a duration of two weeks while the finger millet supplement was given daily for the same duration. The rats were randomly divided into five groups of five rats each (n=5). Group 1 (control) were administered with distilled water (2.5ml/kg), Group 2 were given 6.23mg/kg (3.75%) of HgCl<sub>2</sub> and 100mg/kg of finger millet, Group 3 were given the same dose of HgCl<sub>2</sub> and 50mg/kg of finger millet, Group 4 also received the same dose of HgCl<sub>2</sub> and 25mg/kg of finger millet while Group 5 received HgCl<sub>2</sub> only; all treatments were administered via oral route. After two weeks of administration, rats were euthanized and blood samples were drawn from the heart by cardiac puncture and used to assay for serum urea, creatinine, sodium, potassium, chloride and bicarbonate. Analysis of variance (ANOVA) and Turkey’s post hoc test were used to analyse the data obtained. The result showed a significant (p > 0.05) increase in serum creatinine and urea levels in the HgCl<sub>2</sub> induced groups when compared to the normal control. Similarly, the result showed a significant P>0.05 increase in the serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup>) in the HgCl<sub>2</sub> induced toxicity when compared to the normal control. Furthermore, the result showed a significant P<0.05 decrease in the urea and creatinine levels in the groups treated with 25mg/kg and 50mg/kg when compared to the HgCl<sub>2</sub> induced toxicity group. Similarly, the result showed a significant P<0.05 decrease in the serum electrolytes in the group treated with 25mg/kg and 50mg/kg when compared to the HgCl<sub>2</sub> induced toxicity group. However, the group treated with 100mg/kg showed a decrease in the serum urea, creatinine and electrolytes but was not statistically significant when compared to the HgCl<sub>2</sub> induced toxicity group. The present study suggests that finger millet could be used as a supplement to improve the nephrotoxicity effect induced by mercury chloride.

**Keywords:** Finger Millet, Mercury Chloride, Urea, Creatinine and Serum Electrolytes

### Introduction

Heavy metals such as mercury, when exposed to, pose great health problems, when absorbed, inhaled

or ingested into the bloodstream, they damage tissues like the kidney, brain and liver. Previously, HgCl<sub>2</sub> was used as a disinfectant, laxative, and in the



treatment of depression, and parasitic infections. Today, the use of mercury and its compounds has become limited due to its toxicity, but is still used as a laboratory reagent.<sup>[1,2]</sup> Renal toxicity is one of the significant toxicities, among the confirmed cases. This complication occurs mainly in the proximal tubule. The mechanism of Hg-induced nephrotoxicity is not completely understood, but many studies have found that oxidative stress is an important molecular mechanism for its kidney injury.<sup>[3]</sup> HgCl<sub>2</sub> toxicity causes overproduction of reactive oxygen species (ROS) in the kidney, leading to oxidative damage and cellular dysfunction.<sup>[4]</sup> Morphological and biochemical studies have shown an increase in the production of peroxides such as hydrogen peroxide in renal tubules, decrease in mitochondrial ATP production in cells, and decrease in the activity of free radical scavenging systems (superoxide dismutase and glutathione peroxidase) involved in HgCl<sub>2</sub>- induced nephrotoxicity.<sup>[2,5]</sup> Urea, creatinine, and serum electrolytes, (sodium, Na<sup>+</sup>, potassium, K<sup>+</sup>, chloride, Cl<sup>-</sup> and bicarbonate, HCO<sub>3</sub><sup>-</sup>) are very important parameters for maintaining the body's fluid balance. Their imbalance results in several disorders such as dehydration.<sup>[6]</sup>

Whole-grain consumption is being regarded as a safe approach for the management of toxicity. The consumption of whole millet grains has been shown to be inversely associated with metabolic diseases.<sup>[7]</sup> Finger millet (FM, *Eleusine coracana*) is an important food crop among Africans and Indians. It is regarded as “wonder grain/super cereal” due to health-promoting effects, mainly due to its polyphenols, dietary fibre, lysine and threonine rich proteins and minerals, especially calcium, contents.<sup>[4,5]</sup> FM have been shown to exhibit antioxidant activity<sup>[8]</sup> and in vitro antimicrobial activity;<sup>[9]</sup> inhibit rat intestinal and porcine pancreatic amylase<sup>[10]</sup> and collagen glycation and crosslinking<sup>[10]</sup>; lower plasma glucose concentrations;<sup>[11,12]</sup> improve dermal wound healing;<sup>[13]</sup> and exhibit hypoglycaemic, hypocholesterolaemic, nephroprotective and anti-cataractogenic effects in streptozotocin-induced diabetic rats.<sup>[14]</sup> Due to its enormous health benefits, FM is considered to be a ‘wonder grain’ with superior nutritional qualities.<sup>[15]</sup><sup>[16]</sup> This study was designed to evaluate the effect of finger millet on urea and serum electrolytes in mercury chloride (HgCl<sub>2</sub>) induced toxicity in wistar rats.

## Materials and Methods

### Animals

Twenty-five adult male Wistar rats (obtained from Animal House, College of Medicine, Kaduna State University) weighing 180–200 g, were used for all experiments. The animals were housed in a pathogen-free facility on a 12-hour light/dark schedule, access to food and water *ad libitum*. All animal procedures were approved by the University Ethics Committee and were in compliance with National Laws and with National Institutes of Health guidelines for the use and care of laboratory animals.

### Chemicals

HgCl<sub>2</sub> (mercuric chloride) with a purity of 95% was obtained from Sigma Aldrich. FM was procured from the Agricultural Research Institute, Ahmadu Bello University, Zaria. All other chemicals used were of analytical grade.

### Induction of Experimental Toxicity and Treatment

Toxicity was chemically induced by mercury chloride through oral route of administration on alternate days for a duration of two weeks while the finger millet supplement was given daily for the same duration.

### Experimental design

Twenty-Five (25) adult male Wistar rats weighing 180 – 200 grams were randomly divided into five groups of five rats each (n = 5). Group 1 (control) were administered with distilled water (2.5ml/kg), Group 2 were given 6.23mg/kg (3.75%) of HgCl<sub>2</sub> and 100mg/kg of finger millet, Group 3 were given the same dose of Hgcl<sub>2</sub> and 50mg/kg of finger millet, Group 4 also received the same dose of Hgcl<sub>2</sub> and 25mg/kg of finger millet while Group 5 serve as positive control received 6.23mg/kg of Hgcl<sub>2</sub> only; all treatments were administered once daily via oral route. After two weeks of administration, rats were euthanized and blood samples were drawn from the heart by cardiac puncture. Serum urea was determined by the method described by Marsh *et al.*<sup>[17]</sup> Creatinine was determined by the method described by Bod and Sirota;<sup>[18]</sup> sodium and potassium were measured by the flame photometric method of Vogel.<sup>[19]</sup> Chloride was determined by the method described by Schales and Schales<sup>[20]</sup>

while bicarbonate was measured by the flame photometric method of Segal. [21]

### Statistical Analysis

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

### Results

The results in Table 1 showed significant ( $p > 0.05$ ) increase in serum urea and creatinine levels ( $9.15 \pm 0.81$  mmol/L and  $90.98 \pm 5.52$   $\mu$ mol/L) in the HgCl<sub>2</sub> induced groups when compared to the normal control ( $6.38 \pm 0.35$  mmol/L and  $79.18 \pm 1.90$   $\mu$ mol/L). Similarly, the result in table 2 showed a significant  $P > 0.05$  increase in the serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup>) in the HgCl<sub>2</sub> induced toxicity ( $149.18 \pm 2.28$ ,  $9.3 \pm 0.44$ ,  $114.23 \pm 1.59$  and

$18.25 \pm 0.69$  mmol/L respectively) when compared to the normal control ( $140.72 \pm 6.80$ ,  $6.26 \pm 0.14$ ,  $103.22 \pm 1.62$  and  $15.68 \pm 1.08$  mmol/L respectively). Furthermore, the result in table 1 showed a significant  $P < 0.05$  decrease in the urea ( $4.24 \pm 0.89$  and  $5.37 \pm 0.61$  mmol/L) and creatinine ( $62.12 \pm 4.08$  and  $63.08 \pm 3.22$   $\mu$ mol/L) levels in the groups treated with 25mg/kg and 50mg/kg when compared to the HgCl<sub>2</sub> induced toxicity group. Similarly, the result in table 2 showed a significant  $P < 0.05$  decrease in the serum electrolytes in the group treated with 25mg/kg ( $130.96 \pm 5.61$ ,  $5.08 \pm 0.37$ ,  $104.96 \pm 1.93$  and  $14.46 \pm 1.86$  mmol/L respectively) and 50mg/kg ( $139.15 \pm 1.20$ ,  $4.95 \pm 0.29$ ,  $110.48 \pm 2.73$  and  $15.65 \pm 0.83$  mmol/L respectively) when compared to the HgCl<sub>2</sub> induced toxicity group. However, the group treated with 100mg/kg showed a decrease in the serum urea, creatinine and electrolytes but was not statistically significant when compared to the HgCl<sub>2</sub> induced toxicity group.

**Table 1:** Effect of Varying Doses of Finger Millet on Serum Urea And Creatinine Levels in Mercury Chloride Induced Toxicity.

| Group                           | Urea (mmol/L)     | Creatinine ( $\mu$ mol/L)) |
|---------------------------------|-------------------|----------------------------|
| Control (Distilled water)       | $6.38 \pm 0.35$   | $79.18 \pm 1.90$           |
| HgCl <sub>2</sub> + 100mg/kg FM | $8.63 \pm 0.08$   | $77.88 \pm 1.69$           |
| HgCl <sub>2</sub> + 50mg/kg FM  | $5.37 \pm 0.61^*$ | $63.08 \pm 3.22^*$         |
| HgCl <sub>2</sub> + 25mg/kg FM  | $4.24 \pm 0.89^*$ | $62.12 \pm 4.08^*$         |
| HgCl <sub>2</sub> Only          | $9.15 \pm 0.81$   | $90.98 \pm 5.52$           |

\*denotes statistical significance at  $P < 0.05$  when compared with the control group

FM = Finger millet

HgCl<sub>2</sub> = Mercury chloride (6.23mg/kg)

**Table 2:** Effect of Varying Doses of Finger Millet on Serum Electrolytes Levels in Mercury Chloride Induced Toxicity.

| Group                           | Sodium (mmol/L)     | Potassium (mmol/L) | Chloride (mmol/L)   | Bicarbonate (mmol/L) |
|---------------------------------|---------------------|--------------------|---------------------|----------------------|
| Control(Distilled water)        | $140.72 \pm 6.80$   | $6.26 \pm 0.14$    | $103.22 \pm 1.62$   | $15.68 \pm 1.08$     |
| HgCl <sub>2</sub> + 100mg/kg FM | $141.3 \pm 1.20$    | $5.8 \pm 0.15$     | $105.73 \pm 1.63$   | $14.93 \pm 0.68^*$   |
| HgCl <sub>2</sub> + 50mg/kg FM  | $139.15 \pm 1.20^*$ | $4.95 \pm 0.29^*$  | $110.48 \pm 2.73$   | $15.65 \pm 0.83$     |
| HgCl <sub>2</sub> + 25mg/kg FM  | $130.96 \pm 5.61^*$ | $5.08 \pm 0.37^*$  | $104.96 \pm 1.93^*$ | $14.46 \pm 1.86^*$   |
| HgCl <sub>2</sub> Only          | $149.18 \pm 2.28$   | $9.3 \pm 0.44$     | $114.23 \pm 1.59$   | $18.25 \pm 0.69$     |

\*denotes statistical significance at  $P < 0.05$  when compared with the control group

FM = Finger millet

HgCl<sub>2</sub> = Mercury chloride (6.23mg/kg)

## Discussion

Mercury chloride is a widely used industrial chemical with serious health hazards resulting mainly from environmental pollution by industrial waste or accidental exposure.<sup>[22]</sup>

One of the most important side effects of mercuric chloride in acute or chronic exposure is renal toxicity.<sup>[23]</sup> The most sensitive part of the kidney is proximal tubule. Renal toxicity of mercury is dependent on the destruction time and its density, which can be seen as destruction and vacuolization of microvilli in membrane base and degradation of the cytoskeleton proximal tubules.<sup>[23,24]</sup>

The significantly ( $P < 0.05$ ) higher levels of urea and creatinine in rats treated with mercuric chloride clearly reflected progressing renal damaged induced in the group treated with mercury chloride when compared with the normal control group. In the present study, altered serum urea and creatinine induced by mercury chloride were mostly ameliorated in the groups treated with finger millet supplement with significant difference ( $P < 0.05$ ) in serum urea and creatinine levels in the group treated with 25mg/kg and 50mg/kg when compared to the control group. This is in agreement with the report of Zalups.<sup>[23]</sup>

This effect was dose-dependent, as supported by the report of some researchers that showed decreased serum creatinine level in diabetic rats treated with finger millet.<sup>[25]</sup> A decrease in serum creatinine level usually signifies increased in renal function. Similarly, the significantly ( $P < 0.05$ ) higher levels of serum electrolytes in rats treated with mercuric chloride clearly reflects an altered renal function induced in the group treated with mercury .chloride when compared with the normal control group. Altered serum electrolytes induced by mercury chloride were ameliorated in the groups treated with finger millet supplement with significant difference ( $P < 0.05$ ) in serum electrolytes levels in the group treated with 25mg/kg and 50mg/kg when compared to the control group. However, the group treated with 100mg/kg finger millet supplement did decrease the serum urea, creatinine and electrolytes levels but was not as those of 25mg/kg and 50mg/kg. The study, therefore, recommends that further molecular studies should be carried out to evaluate the possible mechanism of actions in various organ

system and gene expression profile of various enzymes affected by finger millet administration.

## Conclusion

The results of this study showed that finger millet supplementation attenuated  $\text{HgCl}_2$ - induced nephrotoxicity. This is supported by the improvement of serum markers of renal function but explanation and mechanism of this protection need further explorations.

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## Histological Studies on the Effects of Aqueous Seeds Extract of *Datura metel* on the Cerebellar Cortex of Adult Wistar Rats.

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### Abstract

The central role played by the cerebellum in motor coordination and motor learning has long been established and the effect of additives like *Datura metel* (DM) on it needs to be assessed. Thus, the effect of oral aqueous seeds extract of DM on its histology was assessed in an experimental study involving a total of 16 Wistar rats that were randomly placed into 4 groups (groups A, B, C, and D) of 4 rats each. The rats in group A served as the control and were only given distilled-water daily for 2 weeks. The rats in group B, C, and D received 300, 200 and 100 mg/Kg body weights of DM aqueous extract daily for 2 weeks. Histological results revealed that the histoarchitecture of the sections from the control groups of rats were relatively normal in appearance. Dose-dependent histoarchitectural distortion of the H&E stained sections of cerebellar cortex like perineuronal vacuolation, as well as shrinkage of Purkinje cells and generalized cellular degeneration, were observed among the exposed groups of rats. A similar dose-dependent shrinkage and degradation of neuronal fibres was observed in sections of the cerebellum stained with silver stain. In conclusion, the study demonstrated the potentially detrimental effect of DM on the histology of cerebellar cortex in adult Wistar rats.

**Keywords:** Cerebellar Cortex, Silver Staining, *Datura Metel*, Purkinje Layer, Neuronal Degeneration

### Introduction

Medicinal plants are plants that have at least one of their biochemical components or structural parts (Flower, leaves, stem, seeds, barks or roots) been used for therapeutic purposes. <sup>[1]</sup> *Datura metel* (DM) belongs to the family *Solanaceae*, the nightshade, which includes some 2,400 species <sup>[1]</sup>. DM has been documented as a plant with Hallucinogenic properties and the alkaloids present in the plant have been in demand for botanical and medical research. <sup>[2]</sup> The alkaloids content of different parts of the DM plant has been studied and it was observed that the leaves, fruits and roots contain about 38 alkaloids. <sup>[3]</sup> Atropine, hyoscyamine and scopolamine are the main tropane alkaloids. <sup>[4,5]</sup> Megastimane, sesquiterpenes, tannins, phobatanins, cardiac glycosides, carbohydrates, and flavonoids have also been identified in DM extract from its seeds and flowers. <sup>[6]</sup> The hallucinogenic features of DM are believed to be due to its high content of anticholinergic atropine. Thus the delirium, hyperthermia, sedation, tachycardia, bizarre mental confusion, violent behaviour and amnesia that are

characterized by people as feeling “high” are mainly anticholinergic effects. <sup>[2,7,8]</sup> Worldwide, as well as in Nigeria, India and Southeast Asia DM plant commonly grows anywhere as a weed thus making its availability unrestricted. Thus, making it the most abused plant in the world. <sup>[1,9]</sup> There are earlier studies on the effect of DM on some parts of the brain, focusing mainly on the frontal cortex; medial prefrontal cortex, visual pathways, and hippocampus. <sup>[2,10,11]</sup> There is a dearth of literature on the effect of DM on the cerebellum. The aim of this study was to determine the effects of oral administration of aqueous seeds extract of DM on the histology of cerebellar cortex in Wistar rats.

### Materials and Methods

**Materials:** 16 Wistar rats, animal wire mesh cages, sawdust for animal beddings, animals' feeds (Mash grower feeds – Oyo, Nigeria) and distilled water, Whatman's filter paper no. 1, weighing balance (Gallenkamp, UK)

### Experimental Design

Sixteen (16) apparently healthy adult Wistar rats of both sexes and weighing 150-420 g were obtained from the Department of Human Physiology Bayero University, Kano (BUK). The animals were allowed to acclimatize for two weeks at the Department of Physiology Animal house, Bayero University Kano (BUK) and were fed with grower mash and water *ad libitum*. The rats were randomly selected and assigned into four groups A, B, C and D with each group having 4 animals. The rats in Group A served as the control, while those in Group B, Group C and Group D were treated with DM extract.

### Extract Preparation

Fresh fruits of the DM plant were identified and authenticated at the Department of Plant Biology Herbarium, Bayero University, Kano (accession number BUKHAN 0271). The fruits were then macerated at Biochemistry Department, Bayero University, Kano to obtain the seeds; the seeds were air-dried for 21 days under shade. The dried seeds were grinded into powder using a metallic pestle and mortar. The powdered seed material was weighed using Gallenkomp (FA2104A, England) electronic weighing balance; 200 g of the powder was soaked in 1L of distilled water for 72 hrs at room temperature. The filtrate was filtered using a silk material followed by a Whatman's filter paper no. 1 and the residue discarded. The fine filtrate was concentrated using a water bath set at 32 °C and a dark brown sticky mass of the extract was obtained with a total yield of 7.3%.<sup>[12]</sup>

### Animal Treatment

The DM LD<sub>50</sub> of 2640mg/kg as reported in the literature, was adopted in this study<sup>[13]</sup>. Varying dosages of DM used in the study were equivalent to 10%, 7.5% and 5% of the LD<sub>50</sub> for the treatment groups. The rats in group A were given distilled water only while the rats in group B were given 10% of the LD<sub>50</sub>, which was approximated to 300mg/kg-of the extract. The rats in group C and D were given 200mg/ kg-and 100mg/ kg-of the extract. The extract was administered orally to the rats, using an oro-gastric cannula on each treatment day for 14 days, in line with earlier studies.<sup>[14,15]</sup>

### Euthanasia and Brain Dissection

The animals were humanely sacrificed twenty-four hours after the last treatment through cervical decapitation as reported in an earlier study.<sup>[16]</sup> The brain was removed from the skull, and the cerebellum carefully dissected from the brain by cutting through the peduncles. It was then weighed and immediately fixed in a Bouin's fluid.

### Tissue Processing

Brain (cerebellar cortex) tissues were processed routinely for histopathological studies using haematoxylin and eosin staining procedure for demonstrating nuclei and cytoplasm as well as silvers (Bielchowsky) staining techniques for demonstration of nerve fibres. The fixed cerebellar cortex tissue was removed from the fixation medium and dehydrated using ascending grades of alcohol. Dehydrated tissues were then cleared in two changes of xylene for two hours and then infiltrated by immersing in molten paraffin wax and allowed to solidify. The embedded tissues were blocked in a rectangular block. Rotary microtome was used in cutting the tissue sagittally at 10 µm per section. Tissues were allowed to float in a warm water bath of about 30°C to help in spreading the paraffin ribbon, clean glass slides were used to pick up the tissue from the water bath. The histological slides of the cerebellum were mounted on a Motic Photomicroscope fitted with a Celestron© digital microscope imager (USA) with an inbuilt x15 objective lens. The slides were examined and photographed.

### Hematoxylin and Eosin (H and E) Staining Techniques

Cerebral cortex tissues were allowed to dry by dewaxing the tissues in two changes of xylene for 3 minutes each. Descending grades of alcohol; 100%, 95%, 90% and 70% was used to hydrate the tissues for 3 minutes each, stained with Harris haematoxylin for 10 minutes, and washed with tap water to remove excess stain. The tissue slides were flooded with acid alcohol for some seconds for differentiation, washed in tap water again. Bluing was done in water, eosin. Section was washed and used for histological observation.



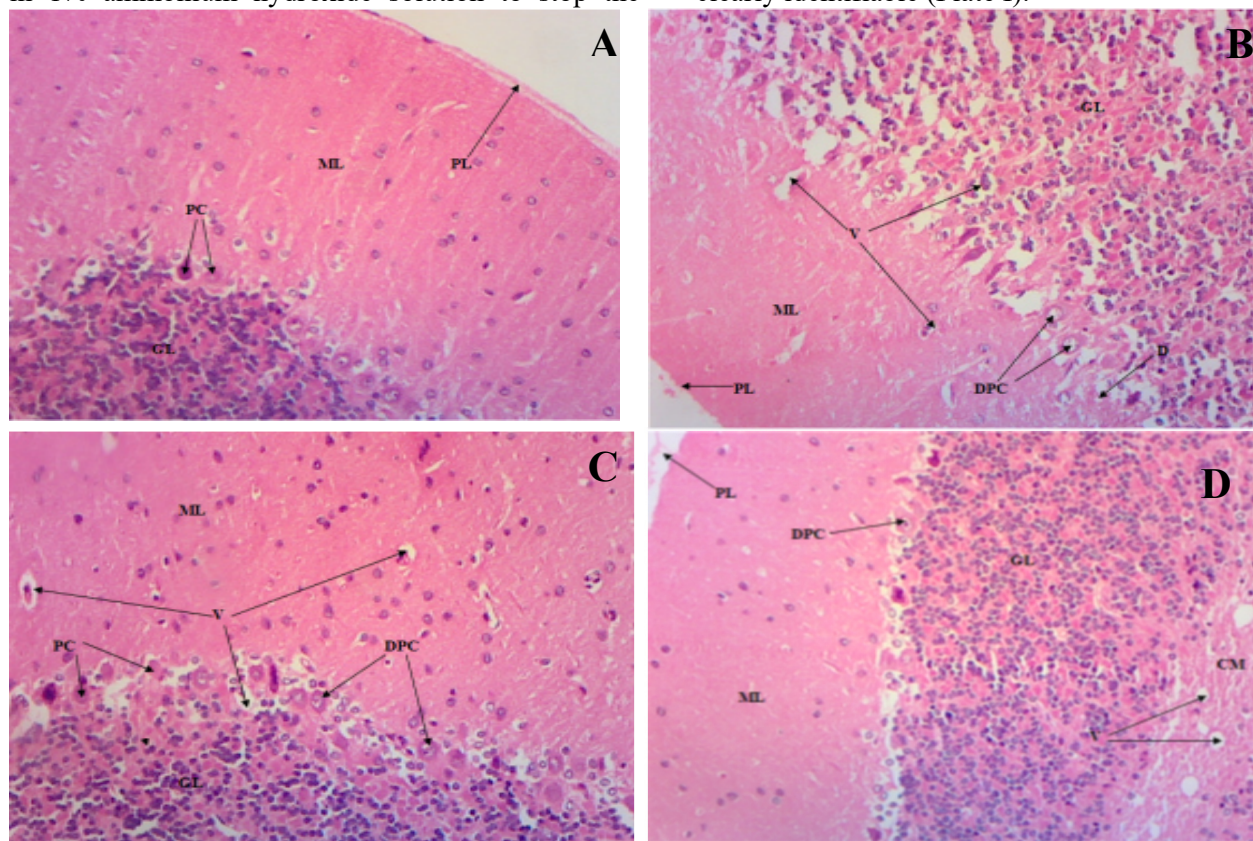
### Silver staining (bialschowsky technique)

The slides were deparaffinized to distilled water and wash three times. The slides were then pre-warmed (40 °C) 10% silver nitrate solution and stained for 15 minutes before placing them in distilled water for wash (3 times). The slides were then placed in ammonium silver solution (made by adding concentrated ammonium hydroxide drop by drop onto silver nitrate solution until precipitates were formed and then few more drops of 10% silver nitrate solutions were added) in 40°C oven for 30 minutes or until sections become dark brown. The slides were then placed in developer working solution for about 1 minute and for another 1 minute in 1% ammonium hydroxide solution to stop the

silver reaction which was then washed off in 3 changes of distilled water. The slides were afterwards placed in 5% sodium thiosulfate solution for 5 minutes and washed in 3 changes of distilled water, dehydrated and cleared through 95% ethyl alcohol, absolute alcohol and xylene.

### Results

The section of the cerebellar cortex of rats from the control groups stained with H&E was observed to be mainly devoid of any abnormality. The structural distinction of the molecular layer (ML) / first layer of the cortex, the granular layer (GL) and the intervening single layer of Purkinje' cells(PC) was clearly identifiable (Plate I).



**Plate 1:** Cross section of Cerebellar Cortex of Wistar rat from the four study groups of oral exposure to saline (control) and varying doses of *Datura Metal*.

A. Section from control animal stained with Heamtoxylin and Eosin at (×150) showing preserved architecture with PL:Pial Layer; GL: Granular Layer; ML: Molecular Layer; PC: Purkinje Cells. B. Section from rat treated with 300mg/kg H and E (×150) showing DPC: Degenerated Purkinje Cells; V: Vacuolation, D: Distortions. C. Section from rat treated with 200mg/kg per day H and E (×150) showing DPC: Degenerated Purkinje Cells; V: Vacuolations. D. Section from rat treated with 100mg/kgbw/day H and E (×150). DPC: Degenerated Purkinje cell; V: Vacuolation.

The cross-section of the cerebellar cortex of adult Wistar rats treated with 300mg/kgbw/day of aqueolayer. The section also had varying levels of Purkinje cells apparent reduction in size, cellular neuronal degeneration (pyknosis), the apparent reduction in size and/or disappearance of some other cells (Plate I).

The cerebellar cortex of adult Wistar rats treated with 200mg/kg/day of aqueous seed extract of DM was observed to have less numerous perineuronal vacuolations in the granular and molecular cell layer (Plate I). The section also had varying levels of Purkinje cells shrinkage, cellular degeneration (pyknosis) with the disappearance of some cells (Plate I).

In the hematoxylin and eosin-stained cross-section of the cerebellar cortex of adult Wistar rats treated with 100mg/kgbw/day of aqueous seed extract of DM few perineuronal vacuolations in the granular and molecular cell layer were observed. Varying levels of Purkinje cells apparent reduction in size, cellular degeneration (pyknosis) with complete disappearance of some cells were also observed (Plate 1). The photomicrograph of the Cerebellum of adult Wistar rat used as the control was shown in Plate II. The Plate showed the molecular layer (ML), Purkinje cells (PC) and granular layer (GL) stained brown, while the fibres in the cerebellar medulla (CM), are stained black.

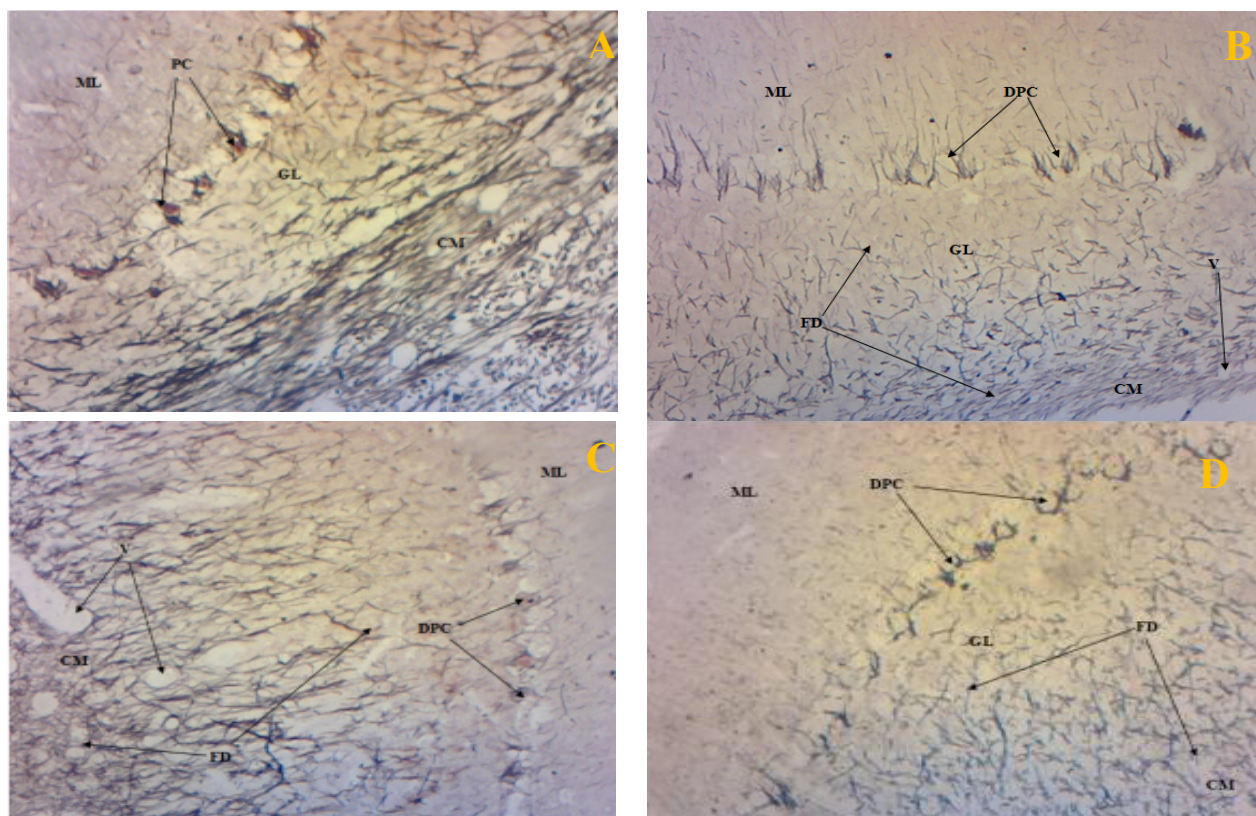


Plate II: Cross section of Cerebellar Cortex of Wistar rat from the four study groups of oral exposure to saline (control) and varying doses of Datura Metal.

A. Section from control animal stained with Silver stain (Bielchowsky) at ( $\times 150$ ) showing preserved architecture **CM**: Cerebellar Medulla; **GL**: Granular Layer; **ML**: Molecular Layer; **PC**: Purkinje Cells. B. Section from rat treated with 300mg/kg Silver stain (Bielchowsky) ( $\times 150$ ) showing **DPC**: Degenerated Purkinje Cells. C. Section from rat treated with 200mg/kg per day Silver stain (Bielchowsky) ( $\times 150$ ) showing **PC**: Purkinje Cells; **DPC**: Degenerated Purkinje Cells; **FD**: Fiber Degeneration; **V**: Perineuronal vacuolations. D. Section from rat treated with 100mg/kgbw/day Silver stain (Bielchowsky) ( $\times 150$ ). **DPC**: Degenerated Purkinje cell; **V**: Perineuronal vacuolation.



In Plate II, the silver-stained cross-section of the Cerebellar cortex of adult Wistar rats from the group that was treated with 300mg/kg/day of aqueous seed extract of DM, it was observed that there was very extensive fibre degeneration in the granular and cerebellar cortex.

In Plate II the silver-stained cross-section of the cerebellar cortex of adult Wistar rats from the group that was treated with 200mg/kg/day of aqueous seed extract of DM, it was observed that there was less extensive fibre degeneration in the granular and cerebellar cortex.

In Plate II, the silver-stained cross-section of the cerebellar cortex of adult Wistar rats from the group that was treated with 100mg/kg/day of aqueous seed extract of DM, it was observed that there were few fiber degeneration in the granular and cerebellar cortex.

## Discussion

There is a dose-dependent distortion in the architecture of the cerebellar cortex and degeneration of the Purkinje cells. These findings in the current study are in agreements to the findings of some earlier studies. [2,11,14] It was reported that the effect of aqueous leaf extract of DM had a deleterious effect on the histoarchitecture of the visual cortex of Wistar rats which was dose dependant. [2] The histoarchitecture changes included perineuronal vacuolations of glial cells and pyramidal cells which were suggestive of imminent cell death. There were also marked perinuclear spacing between neurons as well as an associated reduction in the dimensions of the neurons as evaluated using a stereological grid. [2] These feature reported in the study with the visual cortex especially perinuclear vacuolation as well as the reduction in the dimensions of the neurons were similar to the finding of histoarchitectural changes observed in the current study for effect of DM on cerebellar cortex

Hippocampal granular cell layer of the litters that received 500 mg/kg body weight of DM extract from the 15<sup>th</sup> day of fertilization to parturition had high vacuolations and increased astrocytes density, a clear suggestion of gliosis. [11] The histological section of the medial prefrontal cortex (mPFC) of

Wistar rats treated with DM exhibited dose-dependent features of altered neuronal integrity including chromatolysis, reduced density of Nissl substances, neuronal loss as well as axonal injuries. [14] These histoarchitectural changes reported for both the developing hippocampus and mPFC are in keeping with the findings of neuronal degradation and perineuronal vacuolation observed in the current study.

Thus, the histological changes (perineuronal vacuolations, distortions and cellular degenerative changes) may be as a result of the effects of the phytochemicals (atropine, hyosciamine and scopolamine) as well as carbohydrates and proteins that have been earlier identified in DM. The dose-dependent distortion and atrophy of fibers observed following treatment with DM also suggest that DM has a detrimental effect on the fibres which is characterized as axonal atrophy. [17] Thus, axonal atrophy observed in the extract-treated groups could be an indicator of axonal neurofilaments damage, myelin loss and chromatolysis which are a feature of apoptosis [17]. The current finding regarding the effect of DM on the neuronal fibres is in keeping with the findings of an earlier study that revealed the neuronal fibres degeneration as well as nerve cell edema, chromatolysis following exposure to DM. [1,18]

The precise mechanisms involved in the neurodegenerative effects of DM are mainly hinged on the activities of the alkaloids as well as free radical, contained in the DM extract. The specific mechanism via which the alkaloids promote cellular degeneration appears to involve the increased activities of malondialdehyde (MDA) and superoxide dismutase (SOD) as well as decreased tissue level of glutathione (GSH). [1] Distortion in pathways involved in energy metabolism, homeostasis of neurotransmitters as well as amino acid metabolism disorders have been identified in proton nuclear magnetic resonance (NMR) of the biofluids of mice following exposure to DM and thus, putatively may be associated with its deleterious effect. [1]

It is pertinent to note that the current finding of a dose-dependent distortion of the cytoarchitecture of the different layers and fibres of the cerebellar cortex

reinforce the earlier findings in the literature as regards the distorted cytoarchitecture in other brain regions. The diffuse degeneration of the brain parts that can occur during exposure to DM as well as the high availability and affordability of the extract in the environment increases the need to consider exposure to DM as a public health issue of importance.

## Conclusion

Oral administration of aqueous seed extract of *Datura metel* to in adult Wistar rats produced a varying degree of histological changes in the cerebellum of the rats such as perineural vacuolation, the apparent reduction in neuronal cell size and degenerative changes. These histological changes are indicative of degenerative process in the tissues. *Datura metel* is therefore potentially neurotoxic to the cerebellum in Wistar rats.

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**Conflict of interest:** The authors declare that they have no conflict of interest

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## Histopathological Effects of Repeated In-Utero Ethanol Exposures on Neonatal Cerebral Cortices in Wistar Rats

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### Abstract

Alcohol intake during pregnancy has been shown to cause spectrum of disorders in new-borns which are collectively referred to as Foetal Alcohol Spectrum Disorder (FASD). This results in neuroanatomical malformations, cognitive dysfunction or other behavioural disorders. The aim of the study is to determine the effects of intrauterine ethanol exposure on the histology of the cerebral cortex at varying periods of development in Wistar rats. Fourteen (14) female Wistar rats were mated with sexually matured males in ratio of 2 females to 1 male overnight following determination of pro-oestrous female rats. Pregnant dams were separated into 7 groups of two animals per group. Group A served as the control that received 0.5 ml of distilled water orally throughout gestation. Groups B, C and D were administered daily dose of 0.5 ml of 20% ethanol orally throughout the first week of gestation (Group B), first and second weeks of gestation (Group C) and the whole gestation (Group D). Groups E, F and G were in similar pattern given 0.5 ml of 30% ethanol. The brain tissues of two delivered pups per experimental group were dissected out, fixed in Bouin's fluid and processed for histopathological examination. The histopathological examination of the cerebral cortex of the control animals showed normal cellular architecture. Neurodegenerative changes, namely; pyknosis, satellitosis. The pups exposed to ethanol during first week of gestation and whole gestation period presented similar pattern of pyramidal cell loss and pyknosis, while the pups exposed to ethanol during first and second week of gestation presented clumping of pyramidal cells and pyknosis, the pattern is similar in both 20% and 30% exposed neonates. Degradation of nissl substance was observed in the ethanol-treated groups, 20% ethanol-treated groups exhibited moderately stained nissl substance while 30% ethanol-treated groups showed faintly stained nissl substance, loss of apical dendrites was observed in both the treated groups. Exposure to ethanol during first week of gestation and whole gestation period presented similar pattern of assault on the histoarchitecture of cerebral cortex irrespective of the percentage of the ethanol, while exposure to ethanol during first and second week of gestation irrespective of the percentage of ethanol also presented similar pattern. It can thus be concluded based on the findings of this study that effects of intrauterine ethanol ingestion is more dependent on the period of exposure during embryonic life than the varying percentages of the alcohol.

**Keywords:** *Histochemistry, Histology, Neurodegeneration, Ethanol, Intrauterine.*

### Introduction

Excessive alcohol use accounts for about 5% of global disease burden and close to 6% of all deaths.

[1] It is commonly referred to as booze, bubbly, firewater, joy juice, sauce, liquid courage, and many others. [2] While there are many types of alcohol, the type that is found in drinks and medicines is known as 'ethyl alcohol' or 'ethanol', and it is made by the action of a yeast enzyme changes the simple sugars that are found in grapes, potatoes, or corn into

ethanol<sup>1</sup>. Alcohol is widely recognized as a neuroteratogen.<sup>[3,4,5,6]</sup>

Prenatal alcohol exposure causes several developmental disorders in fetuses with the brain being particularly most affected<sup>7</sup>. Fetal alcohol syndrome (FAS) combines severe symptoms including intrauterine growth retardation, a characteristic craniofacial dysmorphism, central nervous system developmental abnormalities and

behavioral/neurocognitive disturbances<sup>7</sup>. The main cause of acquired, nongenetic mental retardation in industrialized countries is FAS<sup>7</sup>. Neuronal loss is the most devastating consequence of prenatal alcohol exposure which has an age-dependent window of vulnerability, the intrinsic apoptotic pathway via caspase-3 activation mediates the neuronal loss via massive apoptosis<sup>8</sup>. However, it does not affect cell populations in the brain at the same degree, the most damaged structure is the cerebral cortex<sup>9</sup>, pyramidal neurons of layer 5, the main source of output from the cortex, are more susceptible to the death-inducing effects of alcohol, as compared to principal neurons that reside in other cortical layers<sup>9</sup>.

There are literatures suggesting that intrauterine ethanol exposure can lead to epigenetic modification, these emerging evidences suggest that the effects of prenatal ethanol exposure could persist transgenerationally due to tissue-specific epigenetic modifications that pass on to future generations leading to dysfunction in unexposed offspring<sup>10</sup>. There are reports in the literature suggesting that the risk of neuroanatomical malformations, cognitive dysfunction and other behavioural disorders among new-borns were higher in those born by mothers that drink heavily during pregnancy compared to those that don't drink during pregnancy<sup>11</sup>. Preclinical evaluations of the effect of prenatal ethanol exposure included absence of skull vault, under-ossification and asymmetry of the constituent skull bones resulting in severe reduction of the cranial volume in rats<sup>12,13</sup>. It is estimated that the global prevalence of FAS is 0.8% in the general paediatric population<sup>14</sup>, whereas it is as high as 2–5% in the United States<sup>9</sup>. Such high prevalence suggests that it is imperative to understand the mechanisms underlying intrauterine ethanol-induced impairments for scientists and pharmaceutical industries to design effective intervention strategies. The aim of the current study was to evaluate differential histopathological presentation of cerebral cortex of neonatal Wistar rats exposed to ethanol in utero at different periods during gestation.

## Materials and Method

### Experimental Animals

Twenty-one (21) rats were used, comprising of 14 matured female Wistar rats and 7 matured male Wistar rats. The males weighed about 200g-220g

and the sexually matured female rats weighed about 120g-140g at the onset of the study. Animals were housed in wire mesh cages under standard laboratory environment, fed with standard rodent pellets as well as water *ad libitum*. The animals were allowed to acclimatize for 2 weeks prior to commencement of the experiment. The study was conducted in accordance with "Guide for the Care and Use of Laboratory Animals" (<http://www.nap.edu/catalog/5140.html>).

### Oestrous Cycle of the female Animals

Oestrous cycle in Wistar rats last 4-5 days and consists of four main oestrous phases namely: pro-oestrous, oestrous, metoestrous as well as the dioestrous. These phases are characterised by the distinct morphology of the cells found in the vaginal smear. The pro-oestrous phase is predominantly composed of nucleated epithelial cells, the oestrous precedes the pro-oestrous and it is predominated by irregular anucleated cornified cells. The metoestrous preceding the oestrous is composed of three different types of cells, these are: nucleated epithelial cells, irregular anucleated cornified cells, and leucocytes, the last phase which is di-oestrous is predominated by leucocytes<sup>15</sup>. The oestrous phases were determined by collecting the vaginal smear using a soaked cotton bud in distilled water, the soaked cotton bud was then inserted gently in to posterior vaginal wall and manoeuvred gently to obtain some of its contents. The content was then smeared on a clean glass slide and allowed to dry for 1 minute, the dried smear was then stained for 5-10 minutes using 10% giemsa stain.

The stained slides were then washed with Distilled water and allowed to dry for viewing under a light microscope. A maximum of two pro-oestrous female rats were mated with one matured male by placing them in the same cage overnight. Vaginal smear was taken in the morning and the presence of protein coagulate confirmed mating and day zero of pregnancy was designated on such a day<sup>15</sup>. Subsequently, upon confirmation of mating, vaginal smear was taken for three consecutive days to observe if the di-oestrous phase persists, thus, indicating the cessation of ovulation and confirming pregnancy.

### Experimental Design

Following confirmation of mating and pregnancy, the dams were randomly divided into seven (7) groups, each group comprising of two (2) female rats. The dose and the duration of ethanol are summarised in table 1.

### Ethanol Concentration

The concentration of ethanol used in this study was adopted from previous studies. Ethanol was administered via oral intubation as 30% v/v adopted from Adebisi,<sup>16</sup> in addition to lower dose of 20% v/v. 0.5ml as used by Allam and Abdulhamid<sup>6</sup> of the respective doses of the ethanol which was administered orally.

### Ethanol Preparation

100mls of both 20% and 30% ethanol was prepared by diluting 20mls of absolute ethanol in 80mls of distilled water and 30mls of absolute ethanol in 70mls of distilled water respectively.

### Animal Sacrifice

Following parturition, the pups were euthanised using cervical dislocation under ketamine anaesthesia, and the brain tissues obtained were fixed immediately in Bouin's fluid for proper fixation.

### Tissue Processing

The tissues were allowed to stay in Bouin's fluid for 48hrs to enable efficient fixation, and then it was subjected to routine processing for Haematoxylin and Eosin (H and E) and Cresyl Fast Violet staining.

**Table 1:** *Experimental groups and the characteristics of their ethanol administration*

| Groups | Ethanol doses (%)                  | Frequency and Route of administration | Duration                                                         |
|--------|------------------------------------|---------------------------------------|------------------------------------------------------------------|
| A      | No ethanol (0.5ml distilled water) | Orally Once daily                     | 20 Days (Whole gestation)                                        |
| B      | 0.5 ml of 20 %                     | Orally Once daily                     | 7 Days (1 <sup>st</sup> week of gestation)                       |
| C      | 0.5 ml of 20%                      | Orally Once daily                     | 14 days (1 <sup>st</sup> and 2 <sup>nd</sup> weeks of gestation) |
| D      | 0.5 ml of 20%                      | Orally Once daily                     | 20 days (Whole gestation)                                        |
| E      | 0.5 ml of 30%                      | Orally Once daily                     | 7 Days (1 <sup>st</sup> week of gestation)                       |
| F      | 0.5 ml of 30%                      | Orally Once daily                     | 14 Days (1 <sup>st</sup> and 2 <sup>nd</sup> weeks of gestation) |
| G      | 0.5 ml of 30%                      | Orally Once daily                     | 20 Days (Whole gestation)                                        |

## Result

### Histology of the Cerebral Cortex

The histological sections from the experimental animals were all evaluated under a light microscope after appropriate staining. The section from the cerebral cortex of neonatal Wistar rats of the Control Group A have the normal cellular architecture of the cerebral cortex as seen in Figure1(A). The section reveals different types of neural cells including Pyramidal, Fusiform, and stellate cells. The cerebral cortex of neonatal Wistar rat for Group B (received 0.5ml 20% ethanol for 7 days) show karyorhexic, pyknotic, degenerated and clumped cells as shown in Figure1(B).

The cerebral cortex of neonatal Wistar rat for Group C and D that received 0.5ml 20% ethanol for 14 days had a lot of clumped cells and numerous

pyknotic cells as seen in Figure 1 (C and D). The Wistar rat for Group E and G that received 0.5ml 30% ethanol for 7 days and 14 days respectively had similar changes in the sections of their cerebral cortex as those of rats from group B and C as seen in Figure 2 (E and F). Wistar rat from Group G that received 0.5ml 30% ethanol for 20 days had sections replete with vacuolated, Pyknotic and clumped cells (Figure 2G), presenting similar pattern with Group D.

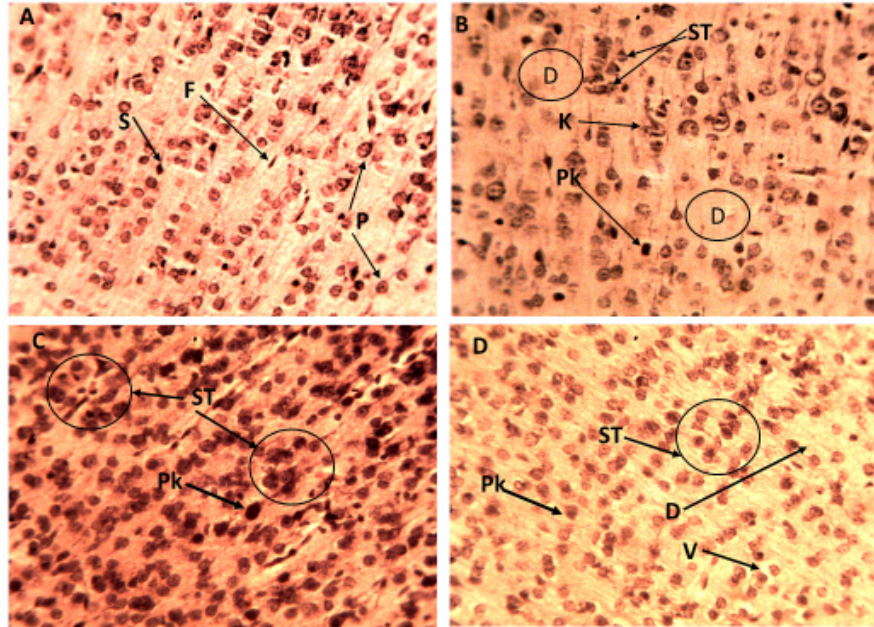
### Histochemistry of Cerebral Cortex

Figures 3A and 4A, demonstrated the cerebral cortex of the Control Group using cresyl fast violet, The section of Cerebral cortex from the animals in the control Group maintain a high density of Nissl substance while the sections from the various ethanol-treated groups exhibited varying levels of

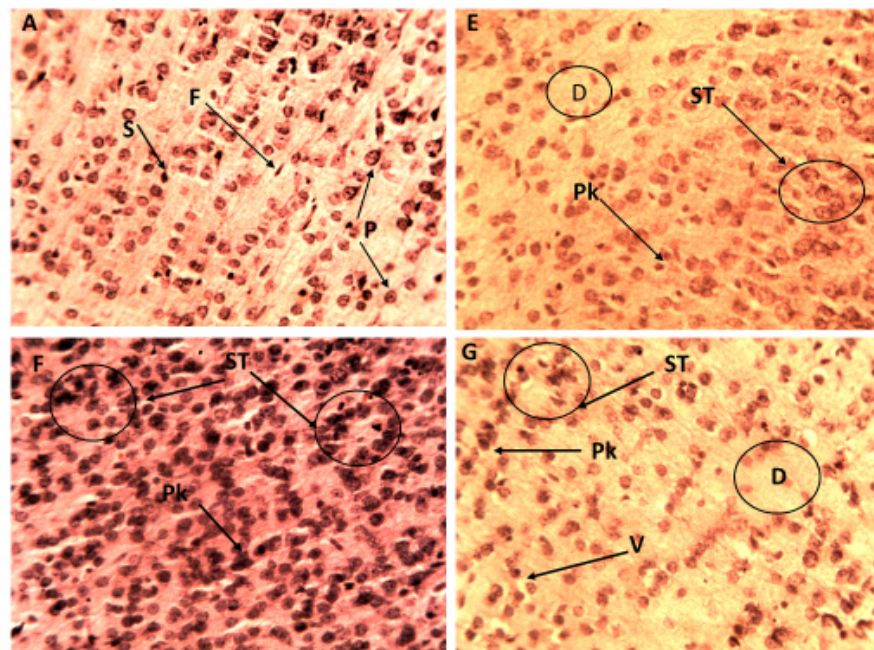


altered Nissl substance density and/or degradation, a – neurocyte chromatolysis (Figure 3A and 4A = control; Figures 3B,C and D and Figures 4E,F and G= treated groups).

Severe neurocyte chromatolysis as seen in sections from animals from group G was also characterised with reduced apical dendrite, distortion in the nuclear outline and neural degeneration.

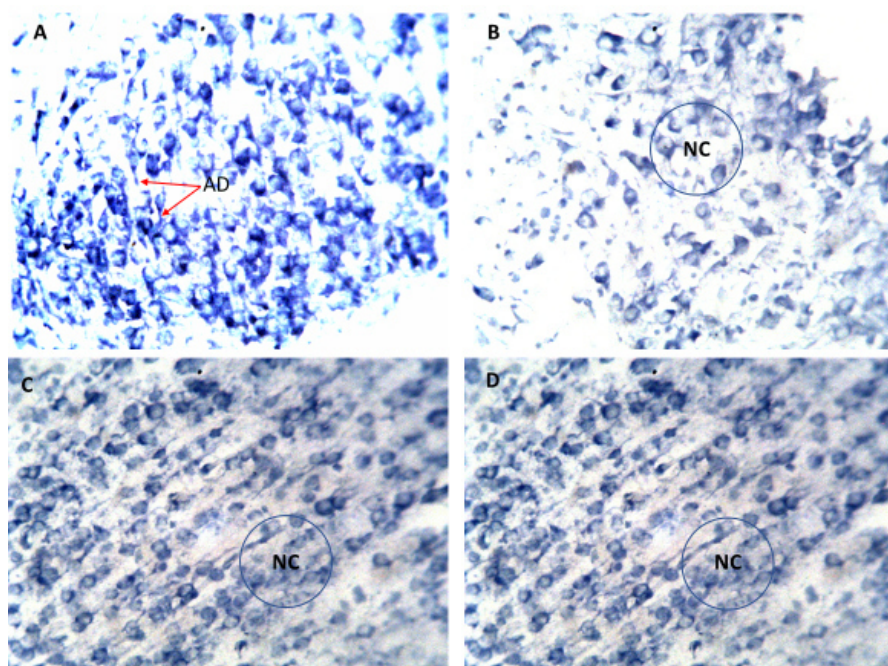


**Figure 1:** A section of Cerebral Cortex. Group A (control) showing normal cellular architecture; Groups B, C and D (ethanol-treated) showing varying histomorphological lesions. Stellate (S), Fusiform (F), Pyramidal (P), Karyorhexic (K), Degenerated (D), Clustered/Satellitosis (ST), Pyknotic (Pk) and Vacuolated (V) cells. (H and E Stain X250)

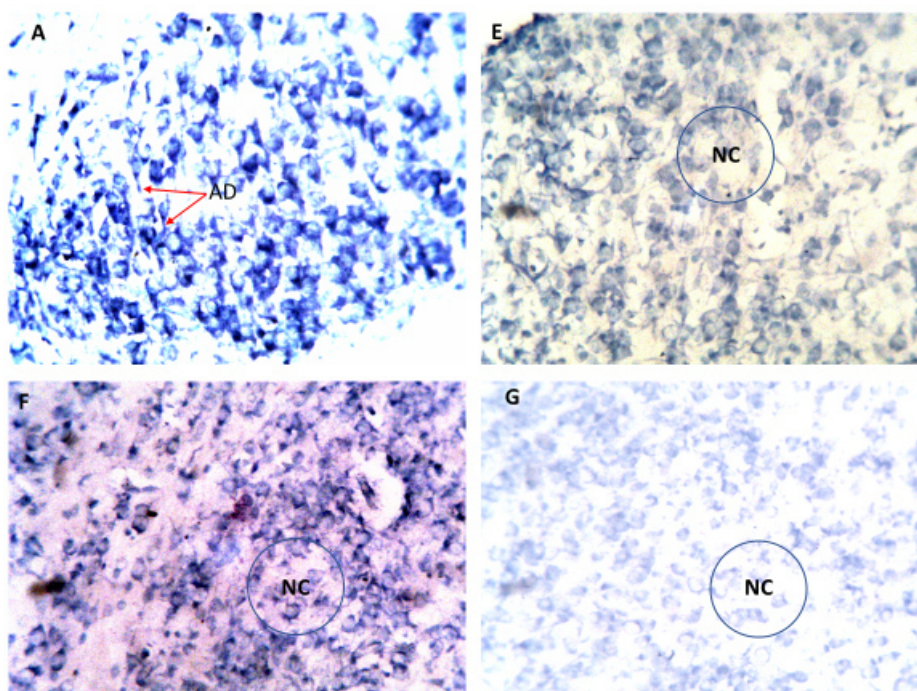


**Figure 2:** A section of Cerebral Cortex. Group A (control) showing normal cellular architecture; Groups E, F and G (ethanol-treated) showing varying histomorphological lesions. Stellate (S), Fusiform (F), Pyramidal (P), Karyorhexic (K), Degenerated (D), Clustered/Satellitosis (ST), Pyknotic (Pk) and Vacuolated (V) cells. (H and E Stain X250)





**Figure 3:** A section of the cerebral cortex. Group A (control) showing highly stained nissl granules with some Apical Dendrites (AD) and ethanol-treated Groups B, C and D showing moderately and faintly stained nissl substance, a feature described as Neurocyte Chromatolysis (NC) (Cresyl Fast Violet stain X250)



**Figure 3:** A section of the cerebral cortex. Group A (control) showing highly stained nissl granules with some Apical Dendrites (AD) and ethanol-treated Groups E, F and G showing moderately and faintly stained nissl substance, a feature described as Neurocyte Chromatolysis (NC). (Cresyl Fast Violet stain X250)



## Discussion

The cerebral cortex of the ethanol-treated neonates revealed varying forms of histopathological and neurodegenerative effects. The effects observed included degeneration of pyramidal cells, pyknosis, chromatolysis, satellitosis and decreased apical dendrite.

The experimental animals exposed to 20% and 30% ethanol during 1<sup>st</sup> week of gestation presented similar pattern of teratologic assault with animal groups that received 20% and 30% ethanol during the whole gestation period. However, vacuolations were observed in the latter in addition to degenerated and pyknotic pyramidal cells observed in all the aforementioned groups.

Satellitosis is a histopathological feature characterized by clustering of cells around dying cells leading to an abnormal increased number of cells. Experimental groups that are exposed to 20% and 30% ethanol during 1<sup>st</sup> and 2<sup>nd</sup> week of gestation were distinctly replete with satellitosis, thus indicating that ethanol exerted teratogenicity in this group irrespective of the percentage of the ethanol but the period and duration of the exposure to the teratogen. The clustered cells can lead to irregular synaptic circuitry that can lead to excitotoxicity or inhibition of cortical functions depending on the types of pyramidal cells and interneurons involved.

The present study revealed marked degeneration of nissl substance in the ethanol-treated groups compared to the control group. This in turn can affect the protein synthesis and metabolism thereby affecting neurotransmission in ethanol exposed neonates. This study also revealed distortion in cellular outline and effects on the pyramidal cells' apical dendrite. There are studies that suggest maternal ethanol ingestion leads to effects on the growth and pruning of dendrites and dendritic spines<sup>17</sup>, this underlying change to the cortical circuitry can result in aberrant neurotransmission and brain activity in areas controlled by the cerebral cortex.

The histopathological effects seen in the present study could result in effects on the electrophysiological activities of the brain due to

abnormal cytoarchitecture of the pyramidal cells observed.

The present study is in agreement with the work of Allam and Abdulhamid<sup>6</sup> which showed that prenatal ethanol exposure led to neurocyte chromatolysis, perivascular oedema, and pyknosis on the section of the cerebral cortex of neonatal Wistar rats. Thus, the effects seen in the present study did not follow a dose-dependent manner, although, the time of the exposure to the teratogen (ethanol) appears to play a key role in the outcome on the cortex and this is in line with an earlier literature report that suggested that the potency of a teratogen depends on the exposure to the teratogen at a critical period of development of the target organs.

## Conclusion

It can be concluded that there is no safe period for alcohol consumption during pregnancy or safe alcoholic concentration for pregnant women. Intrauterine ethanol ingestion at different periods of embryonic life has deleterious effects on pyramidal cells of the cerebral cortex. These effects have varying forms of presentation on the affected neonates, depending on the period of exposure during pregnancy.

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## Identification of SNP in a Master Control Region of SERPIN A1 Gene and its Role in Chronic Obstructive Pulmonary Disease

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### Abstract

Chronic obstructive pulmonary disease (COPD) is a health condition, resulting from both genetic and environmental factors. SerpinA1 is a strong candidate gene in individuals with COPD. It has been suggested that SerpinA1 is regulated by a locus control region (LCR), which activates genes in the proximal serpin sub-clusters by recruiting liver-specific transcription factors and components of the general transcription machinery located between 5-8 kb upstream of the gene. A 461 base pair core element within the master control region is known to be crucial for expression. A total of 177 samples were analyzed (84 COPD and 93 Controls). COPD patients with alpha antitrypsin deficiency were excluded from the COPD samples. One SNP (rs 4905199 A>G) in the LCR region was genotyped, in a pilot study of COPD cases and controls. Oligonucleotides primers were designed to amplify a 638 base pairs region containing the SNP. The study aimed to determine if the genetic variant may confer resistance or protection against COPD. No significant differences were observed with the AA and GG genotypes among the studied and controls. Although, a striking trend was noticed in the control samples, where the AG genotype (25.42%) was significantly higher ( $p < 0.05$ , OR 0.69) than the cases (16.95%). This work suggests that the AG genotype might confer resistance towards COPD although other SNPs in the regulatory region might need to be considered to establish a possible protective role against COPD.

**Keywords:** *SerpinA1*, *Alpha antitrypsin*, *COPD*, *SNP*, *Genotype*.

### Introduction

COPD is a disease that is becoming a global health threat. Its prevalence amongst individuals above 30 years is 10.7% [1] and the third leading cause of death in the United States [2]. The disease is characterized by an irreversible loss of surface area for gaseous exchange as a result of tissue destruction. Genetic and environmental factors contribute to the development of COPD [3]. A genome-wide association study revealed the association of 22 loci linked with COPD [4]. Identification of risk factors and biomarkers could help in targeting screening for those at high risk [5]. The disease is poorly reversible and is characterized by airflow limitation and subsequent decrease of lung function [6].

Alpha Antitrypsin Deficiency (AATD) has been shown to be a strong genetic risk factor for early-onset pulmonary emphysema. Pulmonary

emphysema is a type of COPD that causes the blockage of the airflow leading to breathing difficulties. Alpha Antitrypsin Deficiency (AATD) is also known to be a strong genetic factor for juvenile liver cirrhosis. Smoking is the most important risk factor of non-AATD emphysema [7]. There is evidence that AAT plays a role in early-onset preeclampsia and fetal loss [8] and could be one of the most common single-locus genetic diseases in the world. The manifestation varies in individuals, ranging from asymptomatic in some to fatal disease in others. The underlying mechanisms of tissue loss in emphysema are the protease/antiprotease imbalance as well as increased oxidative stress. [5] Suggested that there is a need to decipher possible protective factors in COPD pathogenesis given that more than half of ever-smokers do not develop COPD. Several reports have suggested the link between AATD and other chronic respiratory

conditions such as asthma and bronchiectasis [9]. This study was aimed at identifying a possible link of an SNP identified in the control region of AAT with the development of COPD.

### **Methodology**

#### **Basic Concepts**

Using valuable resource from Hapmap, a 461 base pair core element was examined. Three SNPs (rs 8008098 T>C, rs10144166 A>G and rs 28694574 T>C) were identified within this core region of less than 5% allele frequencies. Two other SNPs (rs 4905199 A>G and rs 12884390 A>G) were identified very close to this core region having allele frequencies greater than 5%. In this study one SNP, rs 4905199 was genotyped. Oligonucleotides primers were designed to amplify a 638 base pairs region containing the SNP.

#### **DNA Samples**

A total of 177 DNA samples (84 COPD and 93 Controls) were analyzed. COPD patients with alpha antitrypsin deficiency were excluded from the COPD samples. Consent was obtained from the participants.

#### **Polymerase Chain Reactions (PCRs)**

A master mix reaction was prepared containing Taq buffer (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) (Fermentas), reaction buffer containing 2mM MgCl<sub>2</sub> (Roche), 200uM deoxyribonucleotides (dNTPs Fermentas), 1pM of each primer (Forward: 5'-AAAGGCAACCCATTCTTGG-3;' reverse 5'-CCACACTTTCACCAGCACAT-3') using the Eurogentec S.A experimental procedure (Eurogentec Ltd, UK). Taq polymerase (1U) and distilled water were included in the reaction mixture. The reaction was then placed in a thermal cycler (Marshall Scientific, UK) with an initial step at 94°C for 40 seconds followed by 35 cycles consisting of 94°C for 30 seconds (denaturation), 58°C for 1 minute (optimum annealing temperature) and 1 minute at 72°C (for extension) with a final extension step for 10 minutes at 72°C and cooling to 10°C. A blank reaction mixture was used for each experiment to ensure there was no contamination. Optimization of the PCR reaction using temperature gradients (55°C-64°C) was used.

A 1 % (w/v) gel was prepared by dissolving 1 g of agarose powder into 100 ml of tris acetate EDTA buffer, the mixture was shaken vigorously for 30 seconds. The solution was first preheated for 20-30 seconds in a microwave and stirred for a while and then heated again for 10 minutes and allowed to cool to about 65° C in a water bath. Ethidium bromide (2µl) was added into the solution (gel) and the gel was cast into a plate with a comb and allowed to solidify. The solidified gel was inserted into gel electrophoresis tank. DNA ladder (1.5µl) of 1kb was added into the first well. PCR product mixed with 2µl loading dye was added into the remaining wells respectively. The gel was run at 110V and 300 Amp for 35 minutes.

#### **Genotyping (Restriction Digest)**

Following PCR amplification, restriction enzyme was designed using the NEB cutter, Cac8I (Biolabs) was used as the appropriate enzyme. The A allele has one recognition site cutting at 5'-...GCTGGG....3' of 74th base pair position thereby giving two fragments of 74 and 564 base pairs. The G allele has two recognition sites cutting at both 5' ...GCT GGG...3' of the 74th base pair position and at 5'...ACAAGC...3' of 431 base pair position as such producing three fragments of 357, 207 and 74 base pairs. Heterozygotes will then have 4 fragments of 74, 207, 357 and 564 base pairs. Enzymatic reaction was carried in a final volume of 10µl at 37°C for 18hrs and the fragments were separated on a 1% (w/v) agarose gel for 25 minutes at 80V and stained with 3.5µl of ethidium bromide and visualized on an ultraviolet transilluminator. Further assessments of genotypes were done using heterozygous samples as control. All gels were reread blindly by a second person without change.

#### **DNA sequencing**

To confirm the genotyping result, selected samples were examined by DNA sequencing and the results were 100% in concordance. The methods used involve treating the PCR product with Exonuclease (ExoI 10u/µl) to remove primers and shrimp alkaline phosphatase (SAP 1u/ µl) to dephosphorylate excess dNTP. The reaction mixture (2ul) containing exonuclease and alkaline phosphatase was added to a 5µl PCR product. The reaction mixture was incubated in a thermal cycler at 37°C for 15 minutes

with another 15 minutes at 80°C to inactivate the enzyme. The sequence was carried out in MJ Research thermal cycler. Each tube then contains 5ul of cleansed products, 1µl of 5pM/µl primers (primer stocks are at 100pmol/µl) and 4µl of ABI PRISM Big dye Terminator v3.1 Cycle Sequencing (contain fluorescent ddNTPs and enzyme). The reaction was then subjected to thermal cycler at 96°C for 30 seconds with 25 cycles, followed by 50°C for 15 minutes and finally at 60°C for 6 minutes for a total of 25 cycles. Cooling was done for 6 minutes at 4°C. All products were cleaned after the sequencing reaction using gel filtration plate, (Surrey, UK) to

remove excess dye terminators, plates were centrifuged at 2750 rpm for 3 minutes to remove excess buffer. 10µl of water was added to the samples and products applied to a well in the plate and centrifuged at 2750 rpm for 3 minutes. Cleaned samples were then transferred to 0.5 ml tubes for evaporation at 90°C. Finally, sequencing was then analyzed on ABI automated sequencer.

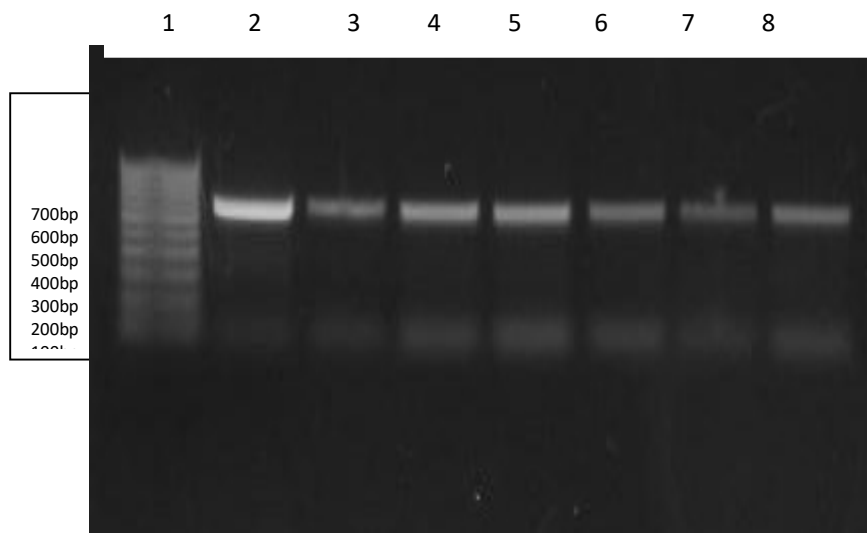
### Statistical Analysis

Allele frequencies will be analyzed using Hardy-Weinberg equilibrium and a pearson chi-square test.

## Results and Discussion

### PCR Optimization

The amplified products gave a size of 638 base pairs as shown in Figure 1.



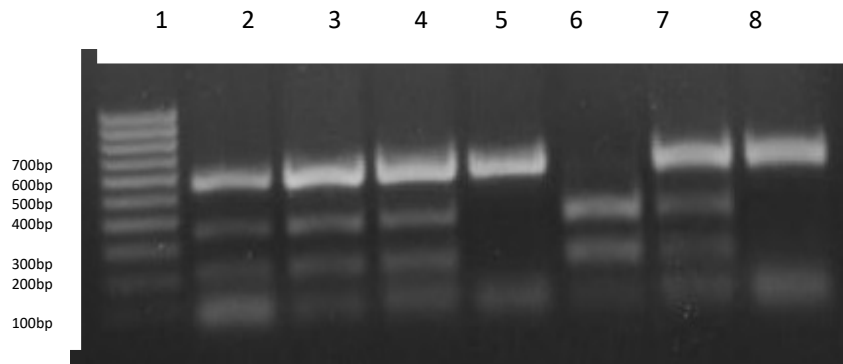
**Figure 1:** Representative agarose gel of some samples stained amplified PCR products:

Lane 1, 100 base-pair marker; lane 2 -8, an amplified product of 638 base-pairs respectively.

### Genotyping

Following DNA amplification of the required region, restriction digest was then carried out to confirm the genotypes of each amplified samples. Basically from the design, the *Cac8I* cuts the fragment size in two places if the genotype of a sample is GG

homozygous, in one place if the sample is AA homozygous, and for heterozygous in both one and two places to produce four bands. Figure 2 indicates some results obtained from the digestion of the PCR products. The digestion gave different bands reflecting the heterozygosity and homozygosity of the genotyped samples.



**Figure2:** Digested samples with different band products; 2, 3, 4 and 7 are heterozygotes (AG), 5 and 8 are Homozygotes wild type (AA) while 6 is homozygotes for the mutant allele (GG). ). Lane 1, 100 base-pair marker. The A allele gives two fragments of 74 and 564 base pairs. The G allele produces 3 fragments of 357, 207 and 74 base pairs. Heterozygotes (AG) have 4 fragments of 74, 207, 357 and 564 base pairs. Heterozygotes (GG) have 2 fragments of 207 and 357 base pairs ►

**Table1.** Genotype frequencies for COPD and Control subjects

| Group   | AA genotype | AG genotype | GG genotype | Total |
|---------|-------------|-------------|-------------|-------|
| COPD    | 36 (20.34%) | 30 (16.95%) | 18 (10.17%) | 84    |
| CONTROL | 31 (17.51%) | 45 (25.42%) | 17 (9.61%)  | 93    |
| Total   | 67          | 75          | 35          | 177   |

The minor allele frequency in the cases was 0.39 while for the control it was 0.42. From the results obtained there was no significant difference in genotypes between the cases and controls. Furthermore, no significant differences were observed with the AA and GG genotype among the cases and controls.

However, a striking trend was noticed in the control samples having the AG genotype (25.42%) significantly higher ( $p < 0.05$ ) than the cases (16.95%). This is an indication that the heterozygous genotype (AG) might have a protective role against the development of COPD. A similar genome-wide association study has reported some SNPs (HHIP: rs1489759, GYPA: rs2202507 and, FAM13A: rs7671167) with the genotype GG, CC, and CC respectively to be higher 17% , 27% and 30% in the controls than the cases 11%, 19% and 23% respectively. The above genotypes were established to be protective against lung cancer and COPD [10].

It is possible that the AG heterozygous genotype in this study might confer resistance towards COPD. This may be implied that the studied AG heterozygous genotype in the control group could be associated with expression or hyper-methylation of  $\alpha$ -1- antitrypsin gene. Since the deficiency or misfolding of this protein is linked to the development of COPD [11]. Further mutational studies conducted on the  $\alpha$ -1- antitrypsin gene has shown the reduced expression of the  $\alpha$ -1- antitrypsin protein due to two identified SNPs rs8004738 and rs28929474, these SNPs were associated with hypo-methylation of SERPIN A1 gene [12]. [13] Argued that  $\alpha$ -1- antitrypsin deficiency is neither a rare nor a disease per se, but rather a potential for a variety of different clinical diseases. It is therefore imperative to understand the effect of genetic polymorphism on the gene and its control region.

### Conclusion

The early diagnosis of COPD using mutational studies might improve clinical outcomes. This work suggests that the AG genotype might confer

resistance towards COPD although, other SNPs in the regulatory region might need to be considered as to establish a possible protective role against COPD. Finally, the genetic variant identified in this work might provide an important and significant contribution to the overall risk score of COPD.

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## Insulin Resistance, Anthropometric Indices And Lipid Profile of Type 2 Diabetic Subjects Attending a Tertiary Health Facility in Kano, North Western Nigeria

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### Abstract

Insulin resistance is central to the development of type 2 diabetes mellitus. Studies have reported an increasing prevalence of dyslipidaemia and obesity in patients with type 2 diabetes mellitus. This study is aimed at studying the relationship between Insulin resistance, obesity indices and lipid profile markers in type 2 diabetes mellitus in a tertiary health facility in Kano, Nigeria. A descriptive cross-sectional design was used to study a random sample of 183 type 2 diabetic patients and 96 controls. Anthropometric, fasting blood glucose, plasma insulin and lipid profile markers were estimated using appropriate techniques. Data were analyzed using SPSS version 23.0. Homeostatic model assessment of insulin resistance (HOMA-IR) score was used to determine insulin resistance (IR). The study observed significantly greater waist-hip ratio (WHR) in the diabetic males than their controls ( $t=2.4$ ,  $p<0.005$ ) while diabetic females had significantly greater body mass index (BMI) ( $t=2.13$ ,  $p<0.005$ ), waist circumference ( $t=4.51$ ,  $p<0.005$ ), hip circumference (HC) ( $t=5.72$ ,  $p<0.005$ ), and WHR ( $t=3.24$ ,  $p<0.005$ ) than their respective controls. Fasting blood sugar (FBS) ( $p<0.005$ ), total cholesterol (TC) ( $p<0.001$ ), low-density lipoprotein (LDL-c) ( $p<0.001$ ) and triglycerides (TG) ( $p<0.005$ ) were significantly higher in the diabetic subjects than the control subjects. However, high-density lipoprotein (HDL-c) ( $p<0.001$ ) was significantly lower in the male diabetic group. The values for IR were greater in both male diabetic subjects ( $1.90 \pm 0.62$ ) than their controls ( $0.97 \pm 0.22$ ) and female diabetic subjects ( $1.81 \pm 0.49$ ) than their controls ( $0.89 \pm 0.19$ ). HC correlated negatively with IR ( $r = -0.205$ ,  $p<0.001$ ) while WHR ( $r=0.218$ ,  $p=0.026$ ) correlated positively with IR in the diabetic female subjects. Markers of dyslipidaemia were found to be higher in the diabetic subjects. Most of the diabetic subjects had IR compared to the control group. IR correlated positively with WHR and negatively with HC in the diabetic female subjects.

**Keywords:** *Insulin Resistance, Anthropometric Indices, Lipid Profile, Type 2 Diabetics, Kano*

### Introduction

Insulin resistance is the inability of insulin to optimally stimulate the transport of glucose into skeletal muscle and adipose tissue, and impair hepatic gluconeogenesis and release of glucose into the circulation.<sup>[1]</sup> Insulin resistance is central in the development of type 2 diabetes mellitus and a core factor in metabolic syndrome.<sup>[2]</sup> This syndrome is a combination of several disorders, which together raise the risk of an individual developing atherosclerotic cardiovascular disease, abnormalities in lipid metabolism and type 2 diabetes mellitus.<sup>[3]</sup> The conditions associated with metabolic syndrome include hyperinsulinaemia, hyperglycaemia, raised blood pressure, elevated blood triglyceride levels, the low blood level of high-density lipoprotein

cholesterol, and obesity (particularly central adiposity/visceral obesity).<sup>[4]</sup> Central obesity (intra-abdominal fat accumulation) is more critical in the development of metabolic disorders like insulin resistance, dyslipidaemia, and high blood pressure; and predisposes more to type 2 diabetes mellitus and cardiovascular diseases.<sup>[5-7]</sup> Visceral fat may contribute to insulin resistance more strongly than subcutaneous fat, although both are known to play a role in the development of metabolic syndrome.<sup>[8]</sup> In central or visceral obesity, high levels of nonesterified fatty acids are released from the adipose tissue causing lipid to accumulate in other parts of the body such as liver and muscle, further perpetuating insulin resistance which could lead to abnormal lipid metabolism.<sup>[9,10]</sup>

Studies have shown a global increase in the prevalence of metabolic disorders arising from insulin resistance, the central factor for metabolic disorders.<sup>[11-18]</sup> Although the pathogenesis for metabolic syndrome from insulin resistance is unclear, recent interest has focused on the involvement of insulin resistance as a linking factor.<sup>[19]</sup> Metabolic disorders have commonly resulted in morbidity and mortality in both developed and developing countries like Nigeria due to lifestyle changes from epidemiologic transition.<sup>[20]</sup> However, measurement of insulin resistance (IR) levels is cost-intensive, and requires sophisticated laboratory investigation which is not accessible for routine outpatient care, especially in low-resource settings.

In order to obtain a proximate and convenient measure of IR for routine outpatient use, this study aimed to study the relationship between insulin resistance, anthropometric indices and lipid profile of diabetic subjects. The study also documents serum levels of these biochemical parameters among diabetic subjects which were hitherto not common in the literature especially among residents of northern Nigeria. Findings from this study would be useful for guiding clinical practice in the management of metabolic disorders, and for further research.

## **Materials And Methods**

### **Study Population and Design**

The study population comprised of type 2 diabetic male and female patients 30 years and above attending the diabetic clinic of Murtala Muhammad Specialist Hospital, Kano, Nigeria. Appropriate controls matched for age, height and weight were selected randomly from healthy non-diabetic subjects in the city. A descriptive cross-sectional design was used and participants were recruited by simple random sampling method.

### **Sample Size Determination**

The sample size was estimated using Fisher's formula for estimating the minimum sample size for descriptive studies.<sup>[21]</sup>

### **Inclusion Criteria**

Type 2 diabetic patients who were 30 years and above.

### **Exclusion criteria**

Hypertensive patients, those with signs of endocrine disorders and chronic illnesses, patients on insulin, and those on other treatments that could affect weight and insulin resistance such as metformin were excluded from the study.

### **Ethical Considerations**

Ethical clearance (protocol no: MAC/SUB/12A/P-3/VI/1075 dated 23rd April 2013) and permission for the study were sought and obtained from the Ethical Committee of Aminu Kano Teaching Hospital, Kano, and the Hospitals Management Board, Kano State, respectively. Informed consent was obtained from each participant before enrolling for the study. The study complied with other relevant guidelines of 2013 Helsinki declaration.

### **Data Collection**

Patients were interviewed using structured interviewer-administered questionnaires. Bathroom scale (Hamason, China) was used to measure the weights of participants, and their heights were measured using a stadiometer (Echukson®, Hamason, China). Participants' hip circumference (HC) was measured using measuring tape at the maximum extension of the buttocks to the nearest 0.1 cm.<sup>[22]</sup> Abdominal (waist) circumference (WC) was measured using the measuring tape at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. The measurement was made at minimal respiration to the nearest 0.1 cm.<sup>[23]</sup>

About 5 ml of blood was drawn into plain bottles from each of the participants between 8 and 10 am after an overnight fast and following all universal precautions. Collected blood samples were centrifuged and stored in a refrigerator at -20°C until all required samples were collected and ready for analysis. Fasting glucose was estimated using glucose oxidase method.<sup>[24]</sup> Fasting insulin level was estimated using enzyme-linked immunosorbent assay (Insulin [Human] ELISA Kit, Taipei, Taiwan) kits, and spectrophotometer EX Lab system was used to measure the absorbance of the samples. The total cholesterol was determined using the enzymatic method, HDL-c was determined using the precipitation method and triglyceride was determined using the enzymatic method.<sup>[25-27]</sup> LDL-

c was determined from the Freidwald's formula:  

$$\text{LDL-c} = \text{TC} - \text{HDL-c} - (\text{TG}/5).$$
 [28]

### Data Analysis

Data were analyzed using SPSS (IBM SPSS Statistics for Windows, version 22. The mean and standard deviation (SD) was used to summarize quantitative variables.

Student's t-test was used to compare quantitative variables, and the Pearson correlation was used to determine the relationship between insulin resistance and anthropometric parameters and lipid profile markers. Homeostasis model assessment of insulin resistance (HOMA-IR) score was used to determine insulin resistance in the study participants, where participants who scored '1' were considered 100% sensitive and those who scored '>1' were considered to have insulin resistance. In this study, HOMA-IR was calculated using the formula:  $\text{HOMA-IR} = (\text{fasting glucose in mmol/L} \times \text{fasting insulin U/L})/22.5$ , where the constant 22.5 is a normalizing factor (normal fasting insulin of 5 U/mL  $\times$  the normal fasting glucose of 4.5 mmol/L typical of a normal healthy individual = 22.5). [28] Body mass index (BMI) was calculated as weight in Kg divided by height in meter squared ( $\text{Kg/m}^2$ ); the waist-hip ratio was calculated as WC in cm divided by HC in cm.

### Results

The subjects comprised of 183 type-2 diabetic patients and 96 non-diabetic controls. More than half (57.4%) of the type-2 diabetic patients were females while the males constituted the remaining 42.6%. The male to female ratio in the group was 1:1.36. On the other hand, 56.2% of the subjects in the non-diabetic group were males and 43.7% were females, with a male to female ratio of 1.28:1. The ages of the type-2 diabetic subjects ranged from 30 years to 81 years, with a mean  $\pm$  (SD) of  $54.09 \pm 12.9$  years. Similarly, the subjects in the non-diabetic group were aged between 30 years and 85 years, and their mean age  $\pm$  SD was  $50.27 \pm 14.42$  years.

Table 1 presents the anthropometric indices of the study subjects. There was no significant difference in the age ( $t=1.81$ ,  $p=0.074$ ), weight ( $t=-0.62$ ,

$p=0.534$ ), height ( $t=0.49$ ,  $p=0.623$ ), BMI ( $t=-0.82$ ,  $p=0.414$ ), WC ( $t=0.49$ ,  $p=0.622$ ), HC ( $t=1.25$ ,  $p=0.213$ ) between the male diabetic subjects and the control subjects. However, significantly higher WHR ( $t=2.34$ ,  $p=0.021$ ) was observed in the diabetic subjects compared to the control. Similarly, there was no significant difference in the age ( $t=1.44$ ,  $p=0.155$ ), weight ( $t=0.75$ ,  $p=0.457$ ) and height ( $t=-1.65$ ,  $p=0.102$ ) between the diabetic female subjects and their control group. However, there was a significant difference in their BMI ( $t=2.13$ ,  $p=0.036$ ), WC ( $t=4.51$ ,  $p=0.001$ ), HC ( $t=2.34$ ,  $p=0.033$ ), and WHR ( $t=3.24$ ,  $p=0.002$ ), and all values were higher in the diabetic group.

When the biochemical parameters were compared between the male diabetic subjects and their control groups, FBS ( $t=12.28$ ,  $p=0.001$ ), cholesterol ( $t=7.25$ ,  $p=0.001$ ), LDL ( $t=8.32$ ,  $p=0.001$ ), triglycerides ( $t=4.20$ ,  $p=0.001$ ) were significantly higher in the diabetic subjects compared to the control subjects. On the other hand, HDL ( $t=-4.72$ ,  $p=0.001$ ) and insulin levels ( $t=-2.15$ ,  $p=0.035$ ) were significantly lower in the diabetic than the control subjects as shown in Table 2. Similarly, FBS ( $t=16.67$ ,  $p=0.001$ ), cholesterol ( $t=3.82$ ,  $p=0.001$ ), LDL ( $t=5.14$ ,  $p=0.001$ ) and triglycerides ( $t=2.13$ ,  $p=0.036$ ) were significantly higher in the female diabetic subjects than the control subjects. On the other hand, HDL ( $t=-5.0$ ,  $p=0.001$ ) was found to be lower in the diabetic than the control groups. However, there was no significant difference in the insulin levels between the female diabetic subjects and the control ( $t=-1.25$ ,  $p=0.215$ ). The prevalence of insulin resistance (using HOMA-IR score of 1 as 100% sensitive) is 97.9% among the diabetic patients and 39.58% among the non-diabetic subjects. Table 2 also shows a statistical significant increase in HOMA-IR in both male diabetic subjects ( $t=12.16$ ,  $p=0.001$ ) and diabetic females ( $t=16.59$ ,  $p=0.001$ ) than the control groups.

Table 3 summarizes the correlation between IR, anthropometric parameters and lipid profile of the subjects. HC ( $r=-0.205$ ,  $p=0.001$ ) correlated negatively while WHR ( $r=0.218$ ,  $p=0.026$ ) positively correlated with IR. However, this study did not find any correlation between insulin resistance and lipid profile markers in both the males and female diabetic subjects.

**Table 1: Anthropometric Indices of Diabetic and Non-Diabetic Participants**

| Parameter               | Male Non-Diabetic (n=54) Mean $\pm$ S.D | Male Type-2 Diabetics (n=78) Mean $\pm$ S.D | P value | Female Non-Diabetic (n=42) Mean $\pm$ S.D | Female Type-2 Diabetic (n=105) Mean $\pm$ S.D | P value |
|-------------------------|-----------------------------------------|---------------------------------------------|---------|-------------------------------------------|-----------------------------------------------|---------|
| Age (years)             | 50.5 $\pm$ 13.4                         | 54.9 $\pm$ 13.4                             | 0.074   | 49.9 $\pm$ 13.90                          | 53.40 $\pm$ 12.1                              | 0.155   |
| Weight (Kg)             | 70.6 $\pm$ 14.                          | 69.1 $\pm$ 12.3                             | 0.534   | 62.45 $\pm$ 12.9                          | 64.21 $\pm$ 12.9                              | 0.457   |
| Height (m)              | 1.7 $\pm$ 0.1                           | 1.7 $\pm$ 0.1                               | 0.623   | 1.57 $\pm$ 0.07                           | 1.54 $\pm$ 0.09                               | 0.102   |
| BMI(kg/M <sup>2</sup> ) | 25.3 $\pm$ 5.0                          | 24.6 $\pm$ 4.3                              | 0.414   | 25.33 $\pm$ 4.30                          | 27.26 $\pm$ 6.34                              | 0.036*  |
| WC(cm)                  | 83.2 $\pm$ 10.4                         | 84.2 $\pm$ 12.2                             | 0.622   | 80.98 $\pm$ 10.5                          | 89.45 $\pm$ 11.9                              | 0.001*  |
| HC(cm)                  | 90.2 $\pm$ 11.3                         | 87.8 $\pm$ 1.13                             | 0.213   | 93.38 $\pm$ 12.4                          | 98.29 $\pm$ 11.7                              | 0.033*  |
| WHR                     | 0.93 $\pm$ 0.08                         | 0.96 $\pm$ 0.07                             | 0.021*  | 0.87 $\pm$ 0.08                           | 0.91 $\pm$ 0.09                               | 0.002*  |

\*significant p value (p&lt;0.05)

**Table 2: Laboratory Parameters of the Diabetic and Control Subjects**

| Parameter                    | Male Non-Diabetic (n=54) Mean $\pm$ S.D | Male Type-2 Diabetics (n=78) Mean $\pm$ S.D | P value | Female Non-Diabetic (n=42) Mean $\pm$ S.D | Female Type-2 Diabetic (n=105) Mean $\pm$ S.D | P value |
|------------------------------|-----------------------------------------|---------------------------------------------|---------|-------------------------------------------|-----------------------------------------------|---------|
| FBS (mmol/l)                 | 4.83 $\pm$ 1.08                         | 9.60 $\pm$ 3.17                             | 0.001*  | 4.46 $\pm$ 0.96                           | 9.16 $\pm$ 2.46                               | 0.001*  |
| Serum Insulin ( $\mu$ IU/mL) | 4.51 $\pm$ 0.17                         | 4.46 $\pm$ 0.07                             | 0.035*  | 4.48 $\pm$ 0.07                           | 4.46 $\pm$ 0.06                               | 0.215   |
| HOMA-IR                      | 0.97 $\pm$ 0.22                         | 1.90 $\pm$ 0.62                             | 0.001*  | 0.89 $\pm$ 0.19                           | 1.81 $\pm$ 0.49                               | 0.001*  |
| Serum TC (mmol/l)            | 4.37 $\pm$ 0.98                         | 5.77 $\pm$ 1.24                             | 0.001*  | 4.26 $\pm$ 0.07                           | 5.10 $\pm$ 1.48                               | 0.001*  |
| Serum TG (mmol/l)            | 1.01 $\pm$ 0.34                         | 1.37 $\pm$ 0.62                             | 0.001*  | 1.11 $\pm$ 0.45                           | 1.29 $\pm$ 0.51                               | 0.036*  |
| Serum LDL-c (mmol/l)         | 2.29 $\pm$ 1.10                         | 3.98 $\pm$ 1.22                             | 0.001*  | 2.19 $\pm$ 1.03                           | 3.31 $\pm$ 1.52                               | 0.001*  |
| Serum HDL-c (mmol/l)         | 1.64 $\pm$ 0.57                         | 1.21 $\pm$ 0.42                             | 0.001*  | 1.68 $\pm$ 0.46                           | 1.27 $\pm$ 0.42                               | 0.001*  |

\*significant p value (p&lt;0.05)

FBS: fasting blood sugar; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol

**Table 3: Correlation between Insulin Resistance, Anthropometric Indices and Some Biochemical Parameters in the Diabetic Subjects.**

| Parameter                | Male diabetic subjects |       | Female diabetic subjects |        |
|--------------------------|------------------------|-------|--------------------------|--------|
|                          | r                      | p     | r                        | p      |
| Height                   | -0.108                 | 0.345 | -0.062                   | 0.532  |
| Weight                   | -0.118                 | 0.302 | -0.168                   | 0.087  |
| BMI (kg/m <sup>2</sup> ) | -0.06                  | 0.546 | -0.111                   | 0.260  |
| WC (cm)                  | -0.098                 | 0.396 | -0.020                   | 0.840  |
| HC (cm)                  | -0.035                 | 0.758 | -0.205                   | 0.036* |
| WHR                      | -0.144                 | 0.209 | 0.218                    | 0.026* |
| Insulin                  | -0.164                 | 0.151 | -0.072                   | 0.383  |
| TC                       | 0.029                  | 0.804 | -0.119                   | 0.226  |
| LDL-c                    | -0.005                 | 0.962 | -0.074                   | 0.454  |
| HDL-c                    | 0.016                  | 0.890 | -0.060                   | 0.545  |
| TG                       | 0.192                  | 0.092 | -0.021                   | 0.835  |

r= correlation coefficient, \*significant p value (p&lt;0.05)

## Discussion

Simple anthropometric measurements have been used as surrogate measurements of obesity and have more practical value in both clinical practices and for large-scale epidemiological studies. The significant higher values of WHR observed in the diabetic male patients and BMI, WC, HC and WHR observed in the female diabetic patients compared to their control subjects is consistent with several investigators who found that measures of both general (BMI) obesity and central obesity (WC and WHR) are associated with a high risk of type 2 diabetes mellitus.<sup>[30-32]</sup> In a longitudinal study done in the USA, among white and black race, the investigators also found that BMI, WC, WHR, WHtR were the best anthropometric discriminators of incident type 2 diabetes mellitus across all race-gender groups in the Atherosclerosis Risk in Communities (ARIC) cohort.<sup>[33]</sup>

Higher levels of insulin among the non-diabetic subjects observed in this study is in consonance with the finding of Bakari, in the study of fasting plasma insulin levels and blood pressure among type 2 diabetic Nigerians.<sup>[34]</sup> The similarity in the findings between the current study and that of Bakari corroborates the possibility that the two study populations may share a causal factor for diabetes mellitus owing to their similarity in ethnicity and environmental factors.<sup>[35]</sup>

The observed higher value of HOMA-IR score in diabetic patients represents greater degrees of insulin resistance in them than the non-diabetic subjects. Reaven *et al.*, in their study “Insulin secretion and action in non-insulin-dependent diabetes mellitus”, also observed that type 2 diabetic patients have lower insulin sensitivity than the non-diabetic group.<sup>[36]</sup> Similarly, Bakari, *et al.*, also reported higher insulin resistance among diabetic patients in Zaria.<sup>[35]</sup> Several other studies have reported higher levels of insulin resistance in diabetic subjects.<sup>[37, 38]</sup>

This study observed higher levels of serum total cholesterol, low-density lipoprotein and triglycerides in the diabetic subjects than in the non-diabetic control, but higher high-density lipoprotein in the control group. The observed higher levels of triglycerides in the diabetic group when compared to that of the non-diabetic group in the study is similar

to that of Bello-Sani *et al.*, from Zaria, Nigeria.<sup>[39]</sup> Similarly, Onyemelukwe *et al.* also from Zaria reported higher levels of triglycerides in the diabetic group than the control.<sup>[40]</sup> The similarity of this finding with that of Bello-Sani *et al.*, and that of Onyemelukwe *et al.*, in Zaria Northern Nigeria, maybe due to the similarities in diet and lifestyle which are among the factors that influence plasma lipids.<sup>[39,40]</sup> Type 2 diabetic patients show high levels of triglycerides because to a large extent hepatic metabolism of triglycerides is controlled by insulin.<sup>[41]</sup> This implies that there is more pool of triglycerides in the blood with inadequate insulin action.

The study also found a positive correlation between insulin resistance and WHR and a negative correlation with HC in female diabetic subjects. This may not be unconnected to the fact that increased visceral adipose tissues measured by WC, WHR are known to increase the risk of metabolic diseases such as diabetes mellitus, hypertension, and dyslipidemia through adverse endocrine and immune pathways. On the other hand, increased subcutaneous adipose tissue which measured hip and thigh circumferences is often described as protective against these disorders.<sup>[42]</sup> Snijder *et al.* reported that large hip and thigh circumferences are associated with a lower risk of type 2 diabetes, independently of BMI, age, and WC, whereas a larger WC is associated with a higher risk of the disease.<sup>[43]</sup> This could also be explained by the fact that the gluteofemoral subcutaneous adipose tissue acts as a metabolic sink entrapping excess free fatty acids.<sup>[44]</sup> Dowling *et al.* suggested that an increase in the lipoprotein lipase activity in the gluteofemoral fat results in an increase in the catabolism of triglycerides and their storage in the adipose tissue which will reduce the concentration of circulating free fatty acids.

They also suggested that the gluteofemoral fat is less lipolytic, releasing less free fatty acids in the circulation and therefore will have less effect in the causation of metabolic diseases compared to the visceral fat since free fatty acids interfere with intracellular insulin signalling and increase hepatic gluconeogenesis and hence insulin resistance.<sup>[45]</sup> Conway, in his study, found that greater HC is associated with increased insulin sensitivity with

reduced insulin concentration in the plasma and hence reduction in the risk of type 2 diabetes mellitus.<sup>[46]</sup>

## Conclusion

Markers of dyslipidaemia were found to be higher in the diabetic subjects. Most of the diabetic patients had insulin resistance (IR) compared with about one-third of the non-diabetic control subjects. IR correlated positively with WHR and negatively with HC in the diabetic female subjects.

## Acknowledgement

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## Conflict of Interest

We declare that none of us has any conflict of interest in this work.

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## Prevalence of *Wuchereria bancrofti* among Human in Tsafe and Talatan-Mafara Local Governments Areas of Zamfara State, Nigeria

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### Abstract

Nigeria is burdened by lymphatic filariasis (LF) caused by the filarial nematode; *Wuchereria bancrofti*. The success of control relies on the identification and treatment of endemic communities. Information on the distribution and nature of the disease from many parts of Nigeria are continuing towards achieving the Global Programme to Eliminate Lymphatic Filariasis (GPELF) by the year 2020. This study was designed to determining the microfilaraemia in human populations of Tsafe and Talatan-Mafara Local Government Areas of Zamfara State, Nigeria. Intravenous blood was examined by microscopy using Thick Blood Film (TBF) method to detect the presence of *Wuchereria bancrofti* of volunteers. The overall result showed that 9 (7.5%) out of the 120 examined were positive. Prevalence was 4.2% in Talatan-Mafara and 3.33% in Tsafe local government areas. The prevalence obtained clearly defined lymphatic filariasis as endemic in both LGAs above the 1% level of endemicity recommended for selecting endemic communities for mass drug administration (MDA) to eradicate the disease.

**Keywords:** Prevalence, Lymphatic filariasis, *Wuchereria bancrofti*, Human, Tsafe, Talatan-Mafara,

### Introduction

Lymphatic filariasis (LF) is a neglected tropical disease (NTDs) caused by the parasitic filaroid nematode worms *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* and transmitted to humans by mosquitoes. <sup>[1]</sup> In Africa, it is caused by *Wuchereria bancrofti* and transmitted by Anopheles and Culex mosquitoes. <sup>[2]</sup> Of the 73 countries where lymphatic filariasis is known to occur, 38 are in Africa and in this region infections are exclusively caused by *W. bancrofti*. <sup>[3]</sup> The third most endemic country for this disease (after India and Indonesia) is Nigeria. <sup>[4]</sup> An estimated 1.34 billion people in 81 countries are at risk of infection by the disease and about 120 million people are infected. About 30% of people at risk reside in the African region while 65% in the South-East Asia Region, and the remainder in other parts of the world. <sup>[1]</sup> The Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched in the year 2000 to interrupt transmission and reduce morbidity and preventing disability. <sup>[2]</sup> Interruption of transmission is possible through mass drug administration (MDA), using once-yearly treatment with a single dose of albendazole plus either ivermectin or diethylcarbamazine (DEC) for

4-6 years. <sup>[2]</sup> Nigeria has the highest number of cases of LF in Africa. About 106 million people in Nigeria are at risk of the disease and 22.1% prevalence of infection. <sup>[5]</sup> The main vectors of LF in Nigeria are mosquitoes of the *An. gambiae s.l.*, *An. arabiensis*, *An. funestus* complexes and *Culex quinquefasciatus*. <sup>[6][7]</sup> Lymphatic filariasis is prevalent in all states and geopolitical zones of Nigeria and a total of 241 lymphodema and 205 hydrocele cases have been reported from mapping surveys conducted in the country. <sup>[8]</sup> The available literature on the disease in Nigeria shows that it is prevalent and widespread in the north-central, northeast, south-east and Niger Delta areas. <sup>[3][9][10][11][12][13][14]</sup>

Independent International Task Force for Disease Eradication to identified lymphatic filariasis as one of only six infectious diseases considered to be “eradicable” with improved tools and methods currently available. <sup>[15]</sup> Nigeria started implementing Mass Drug Administration (MDA) in 2000 in 4 LGAs (Wamba and Akwanga in Nasarawa State and Kanke and Pankshin LGAs in Plateau State) and expanded to 195 LGAs in 2012. Between 2000 and 2012, MDA has been administered to 19,915,586

people. <sup>[16]</sup> Significant progress has been made in the use of MDA for LF control in Nigeria. In order to achieve the goal of LF elimination by 2020, National LF elimination programme of Nigeria needs to scale up MDA in all the states of the federation.

Previous studies have shown that Zamfara State is endemic for a number of tropical diseases, notably schistosomiasis. <sup>[17]</sup> However, the status of lymphatic filariasis in the State is unknown; the prevalence of the disease using parasitological methods in two local government areas of Zamfara State is hereby provided.

## Materials and Methods

### Study Area

Zamfara State is located in the northwestern region of Nigeria. Tsafe and Talatan-Mafara lie between 12°10' - 13° 10' N and 6° 15' - 7°15' E.

The ethnic groups include: Hausa (dominant), Fulani and few of Kanuri. The major economic activities of the people are farming (crop) and rearing of animals, although some are engaged in trading, the Fulani are well known for cattle rearing. The people especially in the rural areas are living in thatched houses virtually unprotected from mosquito bites especially during the hot dry season. Rice paddies especially in Talatan-Mafara provide an ideal breeding site for mosquito vector during the raining season.

### Climate and Vegetation

The vegetation is Sudan savannah characterized by short and scattered trees. <sup>[18]</sup>

Annual rainfall ranges from 36 - 80mm in the months of June to October. The hottest months (March to May) have approximately 40 °C – 45 °C and the coolest months (December to February) have a range of 20 °C – 25 °C. <sup>[18]</sup>

### Ethical Approval

Approval was sought and obtained from the Commissioner of Health through the Director, Department of Public Health, Zamfara State Ministry of Health. Permission was granted by the district heads, community leaders and head of families.

### Collection of Blood Sample

One hundred and twenty (120) samples of blood were collected using venipuncture technique between the hours of 11:00 pm and 12:30 am with the assistance of Medical Laboratory Scientist from each volunteer in the study area in accordance with Center for Disease Control and Prevention (CDC). <sup>[15]</sup> 2ml disposable sterile syringe was used to collect a blood sample from each Individual. The blood samples were immediately transferred to heparinized bottle, closed, mixed thoroughly and labelled. The samples were moved to the correspondent general hospital (Tsafe or Talatan-Mafara Local Government) for further studies.

### Parasitological Examination

The direct wet mount was carried out immediately to observe the motile activity of the positive sample as described by Lynne. <sup>[19]</sup> The thick and thin film was prepared for the detection and speciation of bancroftial parasites respectively. <sup>[20]</sup>

### Procedure

Two to three (2 – 3) drops of blood were placed on a fresh and grease-free slide to make a thick smear and air-dried (18 – 24 hrs.). The dried slides were fixed in 90% methanol for 3 - 4 minutes then later stained in 10% Giemsa solution for 15 - 20 minutes after which removed and washed with buffer solution. Finally, the dried slides were observed under a light microscope (using x40 objective) to assess for the presence of microfilariae as described by the Centre for Disease Control and Prevention. <sup>[15]</sup>

### Results

The prevalence of microfilariae among the human population in the study area is presented in Tables 1 and 2. Of the one hundred and twenty (120) blood samples screened, 9 (7.5%) samples were positive for microfilariae. Out of nine positive cases found, five were from Talatan-Mafara while four from Tsafe Local Government Area. In Tsafe Local Government (Table 1), were recorded in four study sites: Shiyar Magazu 1 (11%), Tudun Muntsira 1 (16%), Kucheri Central 1 (20%) and Ruwa Kusa 1 (14%).

**Table 1:** *Prevalence of Wuchereria bancrofti in human blood in Tsafe Local Government Area, Zamfara State*

| Locations (wards) | Study Sites     | No. examined | No. infected | Prevalence (%) |
|-------------------|-----------------|--------------|--------------|----------------|
| Tsafe Central     | Bakin tumbi     | 12           | 0            | 0.0            |
|                   | Tashar hanne    | 8            | 0            | 0.00           |
| Magazu            | Shiyar Magazu   | 9            | 1            | 11.11          |
|                   | Magajin gari    | 5            | 0            | 0.00           |
|                   | Tudun-muntsira  | 6            | 1            | 16.67          |
| Kucheri           | Sabon-Gari      | 8            | 0            | 0.00           |
|                   | Kucheri Central | 5            | 1            | 20.00          |
|                   | Ruwa-kusa       | 7            | 1            | 14.29          |
|                   | <b>Total</b>    | <b>60</b>    | <b>4</b>     | <b>6.67</b>    |

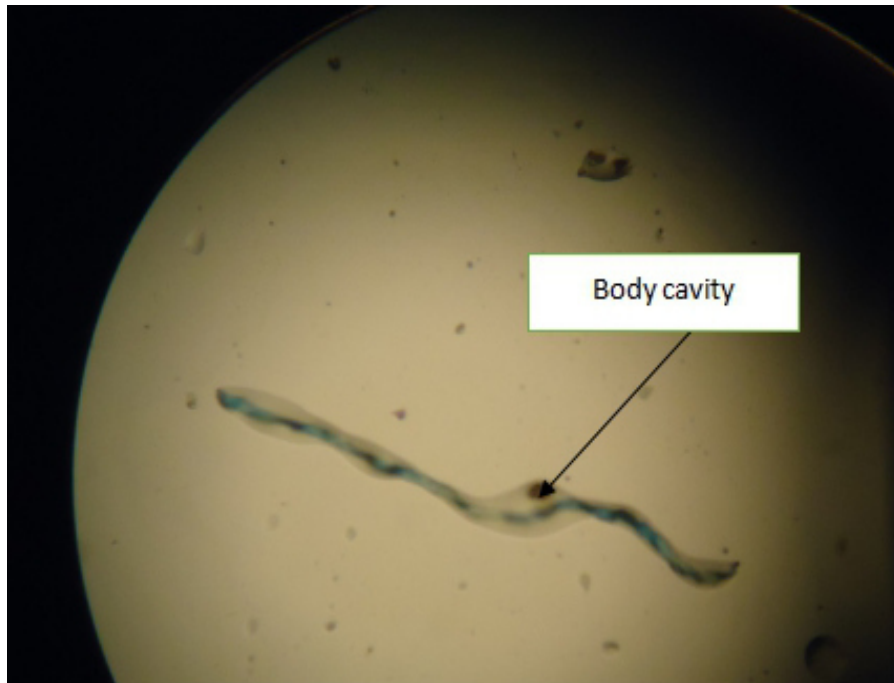
In Talatan-Mafara Local Government Area (Table 2), positive cases were recorded in four study sites: Kwana-Damma 1(11%), Ruwan-Bore 2(18%), Milkidi 1(11%) and Bakin-Zaje 1(9%).

**Table 2:** *Prevalence of Wuchereria bancrofti in human in Talatan-Mafara Local Government Area, Zamfara State*

| Locations (wards) | Study Sites  | No. examined | No. infected | Prevalence (%) |
|-------------------|--------------|--------------|--------------|----------------|
| Galadima          | Kwanan damma | 9            | 1            | 11.11          |
|                   | Anguwan rogo | 5            | 0            | 0.00           |
|                   | Tsakuwa      | 6            | 0            | 0.00           |
| Ruwan-bore        | Ruwan-Bore   | 11           | 2            | 18.18          |
|                   | Milkidi      | 9            | 1            | 11.11          |
|                   | Bakin-zaje   | 11           | 1            | 9.09           |
|                   | Kayatawa     | 9            | 0            | 0.00           |
| Kayayi            | <b>Total</b> | <b>60</b>    | <b>5</b>     | <b>8.33</b>    |

**Table 3:** *Comparison in Prevalence of Lymphatic filariasis in human Between Tsafe and Talatan-Mafara Local Governments*

| Local Govt. Area | Wards         | No. examined | No. infected | Prevalence (%) |
|------------------|---------------|--------------|--------------|----------------|
| Tsafe            | Tsafe Central | 20           | 0            | 0.00           |
|                  | Magazu        | 20           | 2            | 10.00          |
|                  | Kucheri       | 20           | 2            | 10.00          |
|                  | <b>Total</b>  | <b>60</b>    | <b>4</b>     | <b>6.67</b>    |
| Talatan-Mafara   | Galadima      | 20           | 1            | 5.00           |
|                  | Ruwan-Bore    | 20           | 3            | 15.00          |
|                  | Kayayi        | 20           | 1            | 5.00           |
|                  | <b>Total</b>  | <b>60</b>    | <b>5</b>     | <b>8.33</b>    |



**Plate 1:** Showing microfilarial parasite isolated from a human blood sample in Ruwan-Bore during the study

### Discussion

Filarial parasite found in this study was *Wuchereria bancrofti*. The study reveals that people in Talatan-Mafara Local Government Area recorded highest prevalence (8.33%) compared to those in Tsafe Local Government Area (6.67%) and the overall prevalence during the study period was (7.50%). This explains that the two Local Government Areas are endemic with lymphatic filariasis as it above the standard (1% level of endemicity recommended).<sup>[1]</sup> The result showed that people in the rural areas are more infected compare to those who live in Urban and semi-Urban areas of the two Local Governments Areas. Other findings suggest that lymphatic filariasis affects rural more than urban dwellers.<sup>[25][26]</sup> High prevalence (7.50%) reported in this study is consistent with 16.7% obtained in Taraba State, 16.9% recorded in Ebonyi State, 15.49% in Cross River State Nigeria, 3.1% in Dass Area of Bauchi State, and 18.8% observed in Anambra State respectively.<sup>[21][22][23][24]</sup> The high prevalence of LF observed among the Talatan-Mafara and Tsafe people could be as a result of high temperature and humidity of Zamfara that might provide a conducive habitat for mosquito (vector) to breed, hence the parasites' rate of transmission could be high as other findings reveal that temperature and humidity are

part determinant factors to the breeding and density of mosquitoes in a given area.<sup>[28]</sup> Faring (both the rainy and irrigational) and animal rearing are the major occupations in Zamfara State, therefore dams construction for irrigation purpose and animal pens favour mosquito breeding process, these might also contribute toward the transmission of the parasite by mosquito as a vector. No care attitude in compliance to Mass Drug Administration that is ongoing has been observed especial in the rural areas.<sup>[17]</sup> The economic background; which render majority of people in the area live below the poverty line.<sup>[18]</sup> Hence this might result to depress immune of the subjects that could lead to susceptibility to the parasites. In contrast to this finding, low prevalence of lymphatic filariasis were recorded (4.0% and 2.4%) in Ado-Odo Ota and Abeokuta South LGAs of Ogun State respectively.<sup>[29]</sup>

### Conclusion

Despite the ongoing programme by Global Program to Eliminate Lymphatic Filariasis termed: Mass Drug Administration, there was high prevalence of lymphatic filariasis due to *Wuchereria bancrofti* among the study subjects, the study revealed that Talatan-Mafara recorded high prevalence compare to the subjects in Tsafe Local Government Area and

the parasite has more effect in rural compare to urban in the study area.

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## Proximate, Minerals and Vitamins Levels of *Vernonia amygdalina* (bitter leaf), from Maiduguri Borno State, Nigeria

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### Abstract

There is increased awareness in recent years of the health-promoting and protecting properties of nutritive and non-nutrient bioactive compounds found in vegetables. This has directed increased attention to vegetables as vital components of daily diets. The aim of this study, therefore, was to determine the proximate, minerals elements and vitamins of *Vernonia amygdalina* (bitter leaf). The Fresh leafy vegetable was obtained from Maiduguri Monday market and was identified at the Laboratory, Department of Biological Sciences of the University of Maiduguri, Borno State. Standard Laboratory methods of analysis were used to all the analysis. Data collected during the study were subjected to analysis of variances (ANOVA) and means separation using T-Test for the fresh and dried leafy vegetable of *Vernonia amygdalina*. The results revealed that dry matter, crude fibre, ash content crude protein, carbohydrate and energy were high in the dried sample while fats and moisture content were also high in a fresh sample, the results of the minerals and vitamins showed higher values in fresh samples. In minerals composition: The Fresh *Vernonia amygdalina* leaf had the highest value in Na ( $104.21 \pm 0.02 \text{mg}/100\text{g}$ ), Mg ( $62.27 \pm 0.03 \text{mg}/100\text{g}$ ), (P) ( $0.30 \pm 0.012 \text{mg}/100\text{g}$ ), Ca ( $99.58 \pm 0.37 \text{mg}/100\text{g}$ ), Cu ( $3.66 \pm 0.33 \text{mg}/100\text{g}$ ), K ( $163.24 \pm 0.01 \text{mg}/100\text{g}$ ), Iron ( $228.54 \pm 0.60 \text{mg}/100\text{g}$ ) and Zn ( $31.76 \pm 0.04 \text{mg}/100\text{g}$ ). In Vitamins the result revealed that the highest value of Vitamin A ( $18.58 \pm 0.04 \text{mg}/100\text{g}$ ) Vitamin C. ( $65.47 \pm 0.02 \text{mg}/100\text{g}$ ) Vitamin B<sub>9</sub> ( $6.13 \pm 0.01 \text{mg}/100\text{g}$ ). The result showed that fresh *V. amygdalina* leaf had the highest nutritional value in mineral and vitamin and low in dry matter crude protein crude fibre ash content and carbohydrate. This study observed that the fresh *V. Amygdalina* leave studied is a nutritious food that provides some amount of nutrients needed for the maintenance of good health and normal body function.

**Keywords:** *Vernonia amygdalina*, Fresh, Dried Leafy Vegetable, Maiduguri, Nigeria

### Introduction

Nigeria has rich species of plants being used as vegetables and different types are consumed by the various ethnic groups for different reasons. [1,2,3] Vegetables are the fresh and edible portion of plants which can be eaten raw or cooked. [4,5,6] There is increased awareness in recent years of the health-promoting and protecting properties of nutritive and non-nutrient bioactive compounds found in vegetables, which has directed increased attention to vegetables as vital components of daily diets. [7,1,8]

throughout tropical Africa. The bark is green or brownish dark with rough surface and petiole leaves that are about 6 mm in diameter.<sup>[9]</sup> The leaves are elliptical and up to 20 cm long, and also are oblongate lance-like in shape. The leaves are green with characteristic odour and bitter taste. It is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs. [10]

Bitter leaf has been reported to contain the following nutrients: protein, fat, carbohydrate, fibre and minerals which include calcium, sodium, iron, zinc, potassium, magnesium, phosphorus and manganese. [11,12]

*Vernonia amygdalina* (Del.) commonly called bitter leaf is a perennial shrub of 2-5m in height that grows

The objective of the study was to determine the proximate composition, mineral elements and vitamins composition in fresh and dried leaves of *Vernonia amygdalina*. Health practitioners advocated the increase consumption of vegetables, because of their high content of nutritive and non-nutritive dietary components that are safe and more beneficial to man. Some of these vegetables contain minerals and vitamins. However, in north-east Nigeria particularly Borno State, little attention is paid to vegetables that are frequently consumed and due to paucity of knowledge on the nutritional composition of these edible leaves led to this study.

## Materials and Methods

### Sample Collection and Preparation

The fresh leafy vegetables of *Vernonia amygdalina* was obtained from Maiduguri Monday Market and were identified by plant taxonomist at Department of Biological Sciences University of Maiduguri, Borno State. The leaves were rinsed with clean cold tap water, destalked and allowed to drain and then dried. The dried leaves were then weighed into two equal portions of 200g each. The first portion was spread on clean filter paper and kept in a well-ventilated room of the laboratory for two weeks for shade drying and the leaves were constantly turned to avert fungal growth [13]. The second portion 200g of *Vernonia amygdalina* was not subjected to any form of the drying process. After the drying period, the dried leaves were ground into powder using mortar and pestle. The fresh and dried leaves were used for the analyses.

### Determination of Proximate Composition

Proximate Analysis was carried out in both fresh and dry samples of *Vernonia amygdalina* according to the methods of Association Official Analytical Chemist. [14]

### Determination of moisture content (oven drying method)

The moisture content of the samples was determined by weighing 10g of samples into petri dish and placed in a hot oven at 105°C for 2 hours and then removed and placed in a desiccator to cool and then reweighed. The process of weighing and cooling was repeated several times until a constant weight was obtained. The moisture content was calculated using the formula:

Where:

W1: the weight of petri dish with a sample in grams before oven-dried.

W2: weight of petri dish with sample in grams after oven-dried.

W3: weight in grams of an empty petri dish.

### Determination of Dry Matter (DM)

Dry matter =  $100 - \% \text{ moisture content}$

### Determination of Crude Protein

Digestion: Two grams (2g) of the sample was weighed into a digestion tube and one table of Kjeldahl reagent was added. Ten millilitres (10ml) of concentrated  $\text{H}_2\text{SO}_4$  was added to the tube and digested at 420°C for 4 hours. After cooling, 90ml of distilled water was added into the digestion solution.

Distillation: About 50ml of 40% NaOH was added onto 50ml of the digested and diluted solution, and then placed on the heating section of the distillation chamber. Thirty millilitres (30ml) of 4% boric acid, plus bromocresol green and methyl red were also be added as an indicator. This was poured in a conical flask and placed underneath the distillation chamber for collection of ammonia, the solution changed from orange to green colour.

Titration: 0.1 normal solutions of HCl was poured into a burette. The conical flask containing the solution was titrated until the colour changes from green to pink. The burette reading was recorded and the crude protein content was calculated using the formula below:

Where:

A: volume of acid used for titrating the sample

B: volume of the blank sample (0);

N: Normality of acid used for titration

F: Factor 14.007; 6.25: is constant and

100: conversion to percentage

D: aliquot was taken for distillation

CP: Crude Protein

E: weigh of the sample taken for digestion

### Determination of crude fibre

The crude fibre was determined by weighing 1g of sample and placed in a flat bottom flask and 50ml of tri-chloroacetic acid reagent (TCA) was added to the mixture. This were boiled and refluxed for 40 minutes. Filter paper was used to filter the residue:

the residue obtained was washed three times with hot water and once with petroleum ether, then dried to constant weight in an oven at 105°C, cooled in a desiccator and weighed (C1). The weighed sample (C1) was then incinerated in a muffle furnace at 650°C for 2 hours cooled in a desiccator and reweighed (C2). The percentage of crude fibre was calculated from the relation:

The loss in weight on incineration = Crude fibre =  $C1 - C2 \times 100$

#### Determination of ash content

To determine the ash content, 2 g of sample was weighed into a crucible and dried at 105°C for 24 hours, then cooled in a desiccator for 15 minutes and reweighed. It was then charred at 650°C in a muffle furnace for 3 hours, then cooled in a desiccator for 15 minutes and reweighed. Ash content was calculated using the formula:

#### Determination of crude fat

The crude fat was estimated using Soxhlet Apparatus: 2g of the sample was weighed and added into a thimble then covered with cotton wool to prevent spillage. The thimble containing the sample was inserted into soxhlet extractor, the extraction flask was weighed dry and was recorded. The extraction unit was joined with the extractor flask containing 200 ml of petroleum ether. The heater was switched on after being attached to the condenser and was heated at 45°C for 2 hours. The collecting flask was removed and cooled in a desiccator for 15 mins. The percentage of crude fat was calculated using the formula below:

#### Carbohydrate.

Percentage carbohydrate was determined using the formula:

$= 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Protein} + \% \text{ Fibre})$

#### Determination of Energy value

The energy value of the samples was determined by calculation as follows:

#### Mineral Analysis

The method of <sup>[14]</sup> was employed for the determination of mineral content. Sample (5g) of was weighed into a crucible, the crucible with the sample was placed into a furnace and the temperature increased gradually until temperature reaches 550°C. The sample was ashed until white or grey colour was observed in the crucible then the ash was dissolved by adding 2 ml of conc. HNO<sub>3</sub> to the crucible and the dissolved ash was transferred into 100ml volumetric flask and diluted to volume with deionized water and shake thoroughly then used Atomic Absorption Spectrophotometer (AAS) to determine the mineral elements. The absorbance of the solutions was read by Atomic Absorption Spectrophotometer (AAS). The various mineral standards were also prepared to make the calibration curve.

#### Determination of Vitamin Composition

The samples were analyzed for the following: Vitamin A (Carotene), B<sub>1</sub> (Thiamine), B<sub>2</sub> (Riboflavin), B<sub>3</sub> (Niacin), B<sub>6</sub> (Pyridoxine), Vitamin B<sub>9</sub> (folic acid), B<sub>12</sub> (Cyanocobalmine), C (Ascorbic acid), D (Cholecalciferol), E (Tocopherol) and K (phyloquinone) as described by the <sup>[15]</sup> using the Colorimetric methods.

#### Statistical Analysis

Data was collected in e triplicate and were subjected to analysis of variance (ANOVA) means separation using T-Test, at the level of significance (P<0.05).

#### Results

Proximate composition of the fresh and dried sample is presented in Table .1. Significant (P< 0.05) differences were found in the moisture content and fat content of the fresh and dried samples of the *Vernonia amygdalina* leaves with the fresh sample having higher (77.4%) value than the dried sample (8.7%). Higher fat content was observed in the fresh sample (3.72%) compared to the dried (1.20%).

**Table 1:** Proximate composition of fresh and dried *Vernonia amygdalina* leaves.

| Proximate               | Fresh leave             | Dried leave              |
|-------------------------|-------------------------|--------------------------|
| <b>Moisture content</b> | 77.4±0.09 <sup>a</sup>  | 8.7±0.09 <sup>b</sup>    |
| <b>Dry matter</b>       | 22.6±0.08 <sup>b</sup>  | 91.3±0.09 <sup>a</sup>   |
| <b>Crude fibre</b>      | 9.56±0.65 <sup>b</sup>  | 13.22±0.02 <sup>a</sup>  |
| <b>Crude protein</b>    | 7.0±0.03 <sup>b</sup>   | 8.51±0.01 <sup>a</sup>   |
| <b>Crude ether</b>      | 3.72±0.05 <sup>a</sup>  | 1.02±0.01 <sup>b</sup>   |
| <b>Ash content</b>      | 3.46±0.01 <sup>b</sup>  | 5.21±0.03 <sup>a</sup>   |
| <b>Carbohydrate</b>     | 1.38±0.01 <sup>b</sup>  | 62.40±0.03 <sup>a</sup>  |
| <b>Energy kcal/g</b>    | 43.72±0.02 <sup>b</sup> | 301.28±2.01 <sup>a</sup> |

Values are expressed as mean ± SEM. Values with different superscripts within rows are significantly different (P<0.05).

### Mineral Elements Composition

Table 2 shows the mineral element composition of the *Vernonia amygdalina* vegetable. Significant (P < 0.05) differences were found in Na, Mg, K, Ca, and Fe between contents of the fresh and dried samples of the vegetable with the fresh sample having higher values. The fresh values were Sodium (104.21 ±0.02mg/100g), (62.27±0.03mg/100g), (161.47±0.01mg/100g), (98.58±0.37mg/100g) and (186.17±0.01mg/100g) while the dried samples were (84.27±0.03mg/100g), (55.54±0.65mg/100g), (151.46±0.01mg/100g), (96.23±0.03mg/100g) and (142.21±0.01mg/100g) respectively. While in the level of Phosphorus, Zinc and Cupper no significant (p>0.05) difference were observed between the fresh and dried sample of *Vernonia amygdalina* leafy vegetables.

**Table 2:** Mineral Element composition of fresh and dried *Vernonia amygdalina* leaves (mg/100g).

| Mineral Element   | Fresh leave              | Dried leave              |
|-------------------|--------------------------|--------------------------|
| <b>Sodium</b>     | 104.21±0.02 <sup>a</sup> | 84.27±0.03 <sup>a</sup>  |
| <b>Magnesium</b>  | 62.27±0.03 <sup>a</sup>  | 55.54±0.05 <sup>a</sup>  |
| <b>Phosphorus</b> | 0.30±0.02 <sup>a</sup>   | 0.26±0.02 <sup>b</sup>   |
| <b>Potassium</b>  | 161.47±0.03 <sup>b</sup> | 151.46±0.03 <sup>b</sup> |
| <b>Calcium</b>    | 99.58±0.04 <sup>a</sup>  | 96.23±0.03 <sup>a</sup>  |
| <b>Zinc</b>       | 22.70±0.03 <sup>c</sup>  | 22.71±0.01 <sup>c</sup>  |
| <b>Cupper</b>     | 3.66±0.33 <sup>a</sup>   | 3.18±0.032 <sup>a</sup>  |
| <b>Iron</b>       | 168.17±0.03 <sup>c</sup> | 142.21±0.01 <sup>c</sup> |

Values are expressed as mean ± SEM. Values with different superscripts within rows are significantly different (P<0.05).

### Vitamin Composition

The vitamin composition of the *Vernonia amygdalina* leaves is presented in Table 3. Significant (P < 0.05) differences were found in the level of Vitamins A, B<sub>6</sub>, B<sub>9</sub>, B<sub>12</sub> and C between a fresh and dried sample of *Vernonia amygdalina* leaves with the fresh sample having a higher value. The fresh sample had the highest value in vitamin A (18.56±0.04 mg/100g) while dried sample has (12.41±0.02mg/100g). Vitamin C fresh sample has

(65.47±0.02 mg/100g) while dried had (47.23±0.01mg/100g). In B<sub>6</sub> fresh has (2.16mg /100g) while dried had (0.15± 0.01mg/100g). Vitamin B<sub>9</sub> fresh has (6.13 mg /100g) while dried had (4.13 ± 0.01mg/100g). From the result obtained the level of vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, D, E and K did not show significant (P >0.05) differences between fresh and dried samples of *Vernonia amygdalina* leafy vegetable.

**Table 3:** Vitamin composition (mg/100g) of fresh and dried *Vernonia amygdalina* leaves.

| Vitamins           | Fresh leaves            | Dried leaves            |
|--------------------|-------------------------|-------------------------|
| <b>Vitamins A</b>  | 18.58±0.04 <sup>a</sup> | 12.41±0.02 <sup>b</sup> |
| <b>Vitamin B1</b>  | 0.35±0.01 <sup>a</sup>  | 0.34±0.01 <sup>a</sup>  |
| <b>Vitamin B2</b>  | 0.47±0.01 <sup>a</sup>  | 0.44±0.01 <sup>a</sup>  |
| <b>Vitamin B3</b>  | 1.16±0.01 <sup>a</sup>  | 1.14±0.01 <sup>a</sup>  |
| <b>Vitamin B6</b>  | 2.16±0.01 <sup>a</sup>  | 0.15±0.01 <sup>b</sup>  |
| <b>Vitamin B9</b>  | 6.13±0.01 <sup>a</sup>  | 4.13±0.01 <sup>b</sup>  |
| <b>Vitamin B12</b> | 2.06±0.01 <sup>a</sup>  | 1.06±0.01 <sup>b</sup>  |
| <b>Vitamin C</b>   | 65.47±0.02 <sup>a</sup> | 41.47±0.01 <sup>b</sup> |
| <b>Vitamin D</b>   | 0.32±0.01 <sup>a</sup>  | 0.31±0.01 <sup>a</sup>  |
| <b>Vitamin E</b>   | 0.11±0.03 <sup>a</sup>  | 0.10±0.01 <sup>a</sup>  |
| <b>Vitamin K</b>   | 0.06±0.01 <sup>a</sup>  | 0.05±0.01 <sup>a</sup>  |

Values are expressed as mean ± SEM. Values with different superscripts within rows are significantly different (P<0.05).

### Discussion

The results obtained showed a reduction in the moisture content in the dried samples. The high moisture content in the fresh sample was within the range expected for fresh vegetables. [17,18,19]. On the other hand, the result obtained showed a reduction of moisture content in dried samples and this is consistency with other reports. [17,18,19] Dry matter content was low in fresh while in the dried sample was significantly higher (p<0.05).

The fibre content in the dried sample in the present studies correlated with studies of Asaolu *et al.* [11] and [18]. The result of this finding was not in agreement with [19] who recorded fibre content. The difference obtained in this study may be as earlier stated. Leafy vegetables are a good source of fibre which lower the body cholesterol level, consequently, decrease the risk of cardiovascular diseases. It is also required that vegetables should be used frequently as they are good for health and provide all the essential nutrients for normal body functions when consumed in an appropriate combination.

The fat content of fresh and dried was low and in agreement with previous reports. [18] The low crude fat content observed in *Vernonia amygdalina* leaves suggests that the vegetables can serve as part of weight-reducing diet.

Protein content in dried and fresh samples was high compared to the report of Asaolu *et al.*, [11] however it was lower than that reported by other workers. [1,18,19] The present findings indicated the vegetable

contain an appreciable amount of protein which indicates that it can be used for building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones. Proteins also aid in the formation of antibodies that enable the body to fight infection. [18]

Ash content was low compared to the report of Agbaire [17] and Sunday *et al.* [18] The ash content is an indication of the level of inorganic elements such as calcium, magnesium, zinc, potassium, copper and phosphorus in the vegetable. The level of sodium in fresh and dried samples shows that the fresh sample has the highest value and the result obtained in this study was not in agreement with that of Asaolu *et al.* [11] and Sunday *et al.* [18] The difference obtained in this study may be due to soil differences. Variation of sodium and potassium is of significant importance to a hypertensive patient as it enhances blood pressure as revealed by Rehman *et al.* [20]. The magnesium content in the fresh and dried sample was in agreement with Asaolu *et al.* [11] Magnesium can greatly be influenced by the health of the digestive and renal systems. [21] The potassium content in the dry and fresh sample, the result of this finding was not in agreement with the report of Asaolu *et al.* [11] but the result was in a similar range with a report of Ageing *et al.* [6]. The calcium content of the fresh and dried. The results were in a similar range with that reported by Agbaire. [17]

The result from this study showed that the vitamin A content of fresh and dried leaves of *Vernonia*

*amygdalina* varied significantly ( $p < 0.05$ ). The results obtained in this study were in a similar range with the reports of Sarkiyayi and Hamman<sup>[22]</sup> who reported a decrease in the dried sample, which might be due to the drying process. Vitamin B<sub>9</sub> was significantly different ( $p < 0.05$ ), the fresh sample had (6.13mg) and the dried sample had (5.13mg). Vitamin B<sub>9</sub> promote normal digestion essential for development of red blood cells, it also helps in preventing certain birth defects before and during pregnancy. The vitamin B<sub>12</sub> content of the samples was significantly different ( $p < 0.05$ ), the fresh sample has 2.06mg and the dried sample has 1.06mg. The results obtained in this study are in agreement when compared with the reports of Uraku *et al.*<sup>[23]</sup> Vitamin B<sub>12</sub> also help in the formation of red blood cell; it is also important for the healthy nervous system and proper and healthy growth in children. The vitamin C content of fresh and dried leaves varied. The fresh sample had higher vitamin C content which was in agreement with the reports of Ageing *et al.*<sup>[6]</sup> and Uraku *et al.*<sup>[23]</sup> Natural ascorbic acid is vital for the body due to its antioxidant and other metabolic roles.<sup>[24]</sup>

## Conclusion

In conclusion, the results indicated the nutritional value of the leaves of *V. amygdalina*. Additionally, the fresh leaves studied had higher nutritional value than the dried samples.

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## Relationship of Body Mass Index and Head Circumference with Visual Acuity among Undergraduate Student in Kano State, Nigeria

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### Abstract

Overweight and obesity are major public health problems, with increasing prevalence at a fast rate. This study was conducted to determine the relationship between body mass index (BMI), height (H), weight (W) and head circumference (HC) on visual acuity (VA) among apparently healthy students of the Faculty of Basic Medical Sciences, Bayero University Kano. The study was a cross-sectional study in which the data for W, H, HC and BMI measurements were obtained from 400 consenting participants (age 18-35) and analysed. VA was measured using Snellen's chart while anthropometric parameters (AP) were measured using appropriate equipment following standard procedure. The sample consisted of 299 males and 101 females (mean age  $22.11 \pm 2.88$  years). The mean BMI of the study participants was  $20.97 \pm 3.48 \text{ kg/m}^2$ , other mean anthropometric indices assessed included those for HC ( $56.45 \pm 1.67 \text{ cm}$ ), WT ( $59.61 \pm 10.64 \text{ kg}$ ) and HT ( $1.69 \pm 0.07 \text{ m}$ ). The result shows that 89% of the study participants had normal VA while 11% had abnormal VA. The distribution of BMI measurements observed in the current study was normal weight (71.5%), underweight (19.3%), overweight (6.8%) and obese (2.5%) which indicated that majority of the study participants belonged to the normal weight BMI category. The abnormal weight (overweight and obese) participants have a significant decrease in VA when compared to participants in the normal weight BMI category. A linear regression equation for the determination of the right VA was developed based on the current data but not for the left VA.

**Keywords:** Visual Acuity, anthropometric parameters, Prediction, association

### Introduction

The problem of malnutrition has become very common in rural and urban communities because of the difference in socioeconomic status and educational levels. [1-4] Nutritional status is analyzed through the measurement of weight and height using standard procedures and subsequently these measures were used for the calculation of body mass index (BMI). [5] According to (WHO, 2002) people with a BMI  $< 18.5 \text{ kg/m}^2$  were classified as underweight, those with BMI of  $18.5 - 24.99 \text{ kg/m}^2$  were classified as having a normal BMI and those with  $>25 \text{ kg/m}^2$  were classified as overweight BMI. [5] Obesity has become a major worldwide public health problem due to its increasing prevalence (more than 1.9 billion adults worldwide were overweight). [6, 7] Ethnicity and socio-economic status have been identified as two separate independent factors that influence BMI in men and

women. [8,9] Malnutrition have been associated with visual pathologies like diabetic retinopathy, maculopathy and glaucoma that affect visual acuity. Visual acuity (VA) had been described as the ability of an individual to read a standard test pattern at a standard distance and is measured as a ratio of normal vision. [10-15] Nevertheless, the single most common cause of decreased VA is myopia/hypermetropia, and reading the Snellen's chart is the most common test for VA in the clinical setting. [11] Myopia is mainly concerned with the inability to see far objects because the eyeball is excessively longer than normal and/or the cornea is steeply round while the opposite dimensions of the eyeball were associated with hypermetropia. [16 - 18] Thus, dimensions of the head circumference (HC) which may putatively be related to the shape of the head may influence the size of the eyeball viz - a - viz abnormality in visual acuity. [19]

HC is the measurement of the circumference of the head around occiput of the posterior aspect of the

skull to the most anterior portion of the frontal bone. [20, 21] It is an essential part of most pediatric medical

examinations. When it is  $\pm 2$  standard deviation (SD) above or below the reference size of a population for age and sex, it is described as abnormal. [20,22, 23] In children, a clear positive or negative change in the HC percentile position over time suggests hydrocephalus or craniostenosis and there are many syndromes associated with microcephaly and macrocephaly. [20,24] In addition, HC may be associated with height as reported in the literature even though the association with weight is more robust. [25 – 29]

Different approaches have been identified for the determination of VA (Snellen's chart and LogMar chart). The capacity of Snellen's chart to identify the refraction features of good VA (6/5 to 6/12) is similar to that of the logMAR charts as well as the fact that it has no line for assessment of VA score of 6/3 are some of the key peculiarities that enable its usage for rapid screening.<sup>30</sup> The use of pinhole disk to visualize the Snellen's chart reduces the proportion of subjects with false-positive VA results by 55% for scores of less than 20/40. [18] Nevertheless, the false positive rate when evaluating VA after protocol refraction in an eye clinic remains above 20% for letters that can normally be seen at 40mm distance. [18] A test for visual acuity that has high specificity for identifying poor VA has been identified as the protocol refraction that is commonly carried out in the eye clinic as a key ingredient for ophthalmological evaluation. [18] Visual acuity recordings such as, 6/6 depict that the patient is able to see an object that would normally be seen from a distance of 6m (denominator) by people with normal VA while he/she is standing at a distance of 6m (numerator). [30] The challenge with an evaluation of VA is that the entire scope of the testing relies on subjective feedback from patients with attendant consequences to clinical decisions, clinical management and medicolegal issues. [31] There are technically savvy assessments such as enhanced depth imaging optical coherence tomography, computerized optokinetic nystagmus test and visual evoked potentials that have been objectively correlated with the level of VA. [32-34] However, these evaluations are among the highly-priced resources in the field of eye medicine and cannot be applied to the field for day to day screening. In patients with mental retardation and paediatrics (less than 5 years) old, it is a well-known

fact that subjective distance VA evaluation have no place. [32] This caveat may need to be extended further in this era of medicolegal cases to include VA scores that are suggestive of impairment or in cases where foul play is suspected. Thus, approaches that will require any validated objective assessor of VA will form the mainstay of decision in the aforementioned settings.

The search for a non-invasive objective evaluator of VA can advance the goals for highly specific ophthalmological diagnosis. Some studies have evaluated the association between VA and few anthropometric parameters (BMI, height and body fat) and these studies reported that there was an association. [1,10] Nevertheless, statistical evaluation for predictive potentials of these parameters were not carried out in these studies. The prediction of VA from anthropometric parameters (height, weight, BMI and head circumference) among undergraduate students of the Faculty of Basic Medical Sciences of Bayero University, Kano have not been evaluated in the past. The current study aimed to determine the association, as well as the predictive role of some anthropometric parameters (height, weight, head circumference and BMI) for VA among undergraduate students of the Faculty of Basic Medical Sciences. Identifying a role for anthropometric parameters (height, weight, head circumference and BMI) in predicting VA can serve as a quick and cheap screening biological parameter for identifying apparently healthy students and/or poor VA scores that would require extensive ophthalmological assessment for VA.

## **Materials and Methods**

### **Permission and consent for the study**

The entire protocol for the current study was evaluated by the postgraduate board of the Department of Human Anatomy, Faculty of Basic Medical Sciences Bayero University Kano and appropriate suggestions were made to improve on the work. All participants were asked for verbal informed consent before they were recruited into the study.

### **Materials**

The materials used in this study were; Proformer, Measuring tape, Snellen's chart, and stadiometer

### Study area and participants

The study was conducted in the Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University, Kano. The Subjects that participated in this research were undergraduate students from the Faculty of Basic Medical Science College of Health Science, Bayero University, Kano and they were selected using a consecutive sampling technique.

### Inclusion criteria

The study participants included in this study were students of the Faculty of Basic Medical Sciences with apparently normal eyes and no history of eye problem (visual pathology) or head trauma

### Exclusion criteria

The exclusion criteria were mainly centred on students of the Faculty of Basic Medical Sciences that were using lenses for correction of refractive errors or those that were not students of the Faculty of Basic Medical Sciences.

### Study design

The study was an observational cross-sectional study. Detailed determination of appropriate sample size assuming a prevalence of distorted VA of 0.5 (50%) was carried out to allow for the recruitment of adequate number (384) of study participants. Data was obtained through responses of participants regarding socio-demographic features as well as through measurements of weight, height and visual acuity measurement using standard procedures.

### Methodology

Height was measured using a standard stadiometer with the subject standing (without any footwear) in erect posture placing the heel and back against the wall and the readings were taken to the nearest 0.1cm. <sup>[1]</sup> The measurement of weight was done using a calibrated portable weighing machine (Avery), with a total weighing capacity of 120 kg and a sensitivity of 0.05 kg. The weightings were carried out while the participants had minimal clothing on them and stood on the weighing scale in such a way as to exert equal weight on both bare feet. <sup>[1]</sup> Body Mass Index was evaluated as the ratio of weight in kilograms divided by the square of the height in meter (weight (kg)/Height (m<sup>2</sup>)). <sup>[1]</sup> The measurement of head circumference (HC) was done VA in the right eye (Table 1).

using tape rule to the nearest 0.1 cm. Specifically, the tape was placed across the frontal bones just above the eyebrows, around the head and above the ears on each side, while ensuring that it also slips over the occipital prominence at the back of the head. <sup>[20, 22, 23]</sup> The tape was moved up and down over the back of the head to locate the maximal circumference of the head and it was pulled firmly to compress hair and underlying soft tissue. <sup>[20, 22, 23]</sup> Hair ornaments, braids and caps were removed to ensure repeatability for measurement results.

Visual acuity (VA) in each eye was measured using a Snellen's chart. <sup>[1]</sup> The Snellen's chart was hung on a wall at a distance of six (6) metres from the respondent in a well-lit room at a height of two (2) metres. Visual Acuity was measured one eye at a time (monocularly) (bad eye first) with each respondent standing opposite the chart and then reading out the letters on the charts starting from the biggest one to the smallest readable. <sup>[31]</sup> The eye not being measured was covered with a hand-held occlude which was held in place by the researcher. The number of letters the respondents were able to see at this distance was recorded. <sup>[31]</sup>

### Statistical analysis

Age, height, weight, BMI, and VA (expressed numerically) were noted for the whole study population. Mean and Standard Deviation were calculated for age and anthropometric parameters (height, weight and BMI). Detailed bivariate evaluations were carried out using Spearman correlation test for association between continuous variables (between VA and the anthropometric parameters) and Chi-square test for association between categorized versions of study variables (VA: normal and abnormal and BMI: normal, underweight, overweight obese). Level of statistical significance was taken for  $P < 0.05$ . Data were analysed using SPSS version 25.

### Baseline features of study participants

The distribution of the types of VA based on whether it was normal (6/6 (1.00)) or abnormal (6/9;6/12;6/18;6/24 (<1.00)) was determined and 355 (88.8%) of the study participants had normal VA in the left eye while 362 (90.5%) of them had normal

**Table 1:** Frequency Table of Normal And Abnormal Left Visual Acuity.

| Visual acuity |          | Overall<br>(N = 400) | Male<br>(n=299) | Female<br>(n= 101) | Chi square | df | P     |
|---------------|----------|----------------------|-----------------|--------------------|------------|----|-------|
| Right eyes    | Normal   | 362(90.5%)           | 278(93.0%)      | 84(83.2%)          | 8.44       | 1  | 0.004 |
|               | Abnormal | 38(9.5%)             | 21(7.0%)        | 17(16.8%)          |            |    |       |
| Left eyes     | Normal   | 355(88.8%)           | 272(90.7%)      | 84(83.2%)          | 4.64       | 1  | 0.031 |
|               | Abnormal | 44(11.2%)            | 28(9.3%)        | 17(16.8%)          |            |    |       |

The high proportion of participants with normal VA was maintained irrespective of the gender of the study participant even though there were 299 (74.8%) males and 101 (25.3%) females in the study (Table 1). The evaluation of the distribution of study

participants based on the WHO classification of body mass index revealed that 286 (71.5%) participants had a BMI that was classified as normal while 77 (19.3%) were underweight, 27 (6.8%) were overweight and 10 (2.5%) were obese (Table 2).

**Table 2:** Frequency and Relationship of BMI Categories with Gender and Visual Acuity in Each Eye.

| BMI           | Overall    | Male<br>(n=299)         | Female<br>visual acuity<br>(n = 101)<br>abnormal (45) | Right visual acuity     |                         | Left                    |
|---------------|------------|-------------------------|-------------------------------------------------------|-------------------------|-------------------------|-------------------------|
|               |            |                         |                                                       | normal (n= 362)         | abnormal (n=38)         | normal (355)            |
| Underweight   | 77(19.3%)  | 48(16.1%)               | 29(28.7%)<br>12(27.3%) <sup>f</sup>                   | 65(18.0%)               | 12 (31.6%) <sup>c</sup> | 64(18.0%) <sup>f</sup>  |
| Normal weight | 286(71.5%) | 230(76.9%) <sup>a</sup> | 56(55.4%) <sup>a</sup><br>22(50.0%) <sup>c</sup>      | 270(74.6%) <sup>b</sup> | 16 (42.1%) <sup>b</sup> | 264(74.4%) <sup>c</sup> |
| Overweight    | 27(6.8%)   | 18(6.0%)                | 9(8.9%)<br>4(11.3%) <sup>g</sup>                      | 23(6.4%) <sup>d</sup>   | 4 (10.5%) <sup>d</sup>  | 23 (6.5%) <sup>g</sup>  |
| Obese         | 10(2.5%)   | 3(1.0%)                 | 7(6.9%)<br>6(13.6%)                                   | 4(1.1%)                 | 6 (15.8%)               | 4(1.1%)                 |

a: Logistic regression with Obese group as reference; evaluation based on gender = Normal weight group female were significantly more than the normal weight females (P = 0.001; OR 0.104, CI = 0.026 – 0.416); b: evaluations based on visual acuity = Among participants with normal weight, participants with normal visual acuity in the right eye were significantly more than those with abnormal VA in the same eye (P = 0.000; OR=25.31, CI = 6.48 – 98.82); c: among participants with underweight, participants with normal visual acuity in the right eye were significantly more than those with abnormal VA in the same eye (P = 0.004; OR=8.13, CI = 1.989 – 33.18); d: among participants with overweight, participants with normal visual acuity in the right eye were significantly more than those with abnormal VA in the same eye (P = 0.011; OR= 8.63, CI = 1.654 – 44.99). For the evaluations based on visual acuity = e: among participants with normal weight, participants with normal visual acuity in the right eye were significantly more than those with abnormal VA in the same eye (P = 0.000; OR= 18.00, CI = 4.72 – 68.59); f: among participants with underweight, participants with normal visual acuity in the left eye were significantly more than those with abnormal VA in the same eye (P = 0.004; OR=8.00, CI = 1.96 – 32.68); g: among participants with overweight, participants with normal visual acuity in the left eye were significantly more than those with abnormal VA in the same eye (P = 0.011; OR= 8.63, CI = 1.654 – 44.99).

The distribution of the different types of BMI among the study participants was essentially the same for participants that were males and those with normal right and left eye visual acuity (Table 2). The high proportion of male participants with normal BMI (n= 230) was significantly (P = 0.001; OR 0.104, CI = 0.026 – 0.416) more than the number of female participants with normal BMI (n = 56). There were significantly more participants that were underweight (P = 0.004; OR=8.00, CI = 1.96 – 32.68), normal weight (P = 0.000; OR= 18.00, CI = 4.72 – 68.59) and overweight (P = 0.011; OR= 8.63,

CI = 1.654 – 44.99) that had normal VA compared to those with abnormal VA in the left eye. The association between the participants with normal weight and VA (with results from the patients in the obese BMI category serving as reference) was more robust in both the left eye (P = 0.000; OR= 18.00, CI = 4.72 – 68.59) as well as the right eye (P = 0.000; OR=25.31, CI = 6.48 – 98.82) (Table 2). The mean anthropometric statistics among undergraduate student of the Faculty of Basic Medical Sciences, Bayero University Kano, were highlighted in Table 3.

**Table 3:** The mean of age and other measured anthropometric parameters among students of Faculty of Basic Medical Sciences, Bayero University Kano

| Variables                | Overall<br>(N=400; 100%)<br>Mean ± SD | Male<br>(n = 299; 74.7%)<br>Mean ± SD | Female<br>(n = 101; 25.3%)<br>Mean ± SD | Z       | P                        |
|--------------------------|---------------------------------------|---------------------------------------|-----------------------------------------|---------|--------------------------|
| Age (year)               | 22.11±2.88                            | 22.61 ± 2.78                          | 20.64 ± 2.67                            |         |                          |
| HC (cm)                  | 56.45±1.67                            | 56.24 ± 1.47**                        | 57.08 ± 2.05**                          | -3.537  | 0.0004                   |
| H (cm)                   | 169±7.00                              | 171 ± 7.00**                          | 161 ± 6.00**                            | -11.068 | 1.785 x10 <sup>-28</sup> |
| WT (kg)                  | 59.61±10.64                           | 61.07 ± 8.77**                        | 55.31 ± 14.06**                         | -6.554  | 5.609x10 <sup>-11</sup>  |
| BMI (kg/m <sup>2</sup> ) | 20.97±3.48                            | 20.87 ± 2.74                          | 21.28 ± 5.08                            |         |                          |

H= Height WT= weight, HC=head circumference, BMI=body mass index, Note \*\* = P ≤ 0.001

The distribution of anthropometric measures (height, head circumference and weight) was different when evaluations were done along gender lines. The mean age (22.11±2.88 years), head circumference (56.45±1.67 cm), height (1.69±0.07 m), weight (59.61±10.64 kg) and BMI (20.97±3.48 kg/m<sup>2</sup>) were identified for the study participants in the current study. The mean values for the anthropometric parameters observed after categorizing the anthropometric measurements based on gender were

similar to those observed when the population was not categorized (Table 3). Nevertheless, there was a statistically significant difference in the distribution of the measurements for HC (P = 0.0004), height (P = 1.785 x10<sup>-28</sup>) and weight (P = 5.609x10<sup>-11</sup>) among the males when compared to the female study participants (Table 3). Anthropometric parameters were further evaluated based on the type of VA recorded in the right and left eye of the study participants (Table 4).

**Table 4:** Anthropometric parameters based on the type of visual acuity measured for the right and left eyes

| Variables                | Mean±SD       |                 |        |             |               |                 |        |          |
|--------------------------|---------------|-----------------|--------|-------------|---------------|-----------------|--------|----------|
|                          | Normal<br>LVA | Abnormal<br>LVA | (Z)    | P-<br>value | Normal<br>RVA | Abnormal<br>RVA | (Z)    | P-value  |
| HC (cm)                  | 56.48 ± 1.66  | 56.22 ± 1.83    | -0.599 | 0.549       | 53.48 ± 1.64  | 56.18 ± 1.97    | -0.963 | 0.336    |
| H (m)                    | 1.69 ± 0.07   | 1.66 ± 0.08     | -2.534 | 0.011       | 1.69 ± 0.08   | 1.64 ± 0.06     | -3.996 | 0.000064 |
| WT (kg)                  | 59.49 ± 9.43  | 60.62 ± 17.61   | -1.116 | 0.265       | 59.51 ± 9.39  | 60.65 ± 18.97   | -1.579 | 0.114    |
| BMI (kg/m <sup>2</sup> ) | 20.83 ± 2.95  | 22.64 ± 6.17    | -0.474 | 0.636       | 20.81 ± 2.94  | 22.52 ± 6.57    | -0.055 | 0.956    |

Values are mean ± standard deviation, H= height, WT= weight, HC= head circumference, BMI=body mass index, LVA= left visual acuity, RVA= right visual acuity.

The distribution of anthropometric measures (height, head circumference and weight) was different when assessed along gender lines while only the distribution of height was different based on the type of VA in the right and left eye. The participants' height (P = 0.011) was significantly higher among those with normal left VA compared to those with abnormal left VA (Table 4). Similarly, only the distribution of height (P = 0.000064) was significantly different among those with normal right

VA compared to those with abnormal right VA (Table 4).

#### Linear regression evaluations

The stepwise logistic regression for the evaluation of the association between the anthropometric parameters and VA in the right eye suggested that the height and intercept model (Model 1) was the only model for the prediction of VA and the results were very robust (F = 13.291, P = 0.00032)(Table 5).

**Table 5:** Stepwise linear regression for the association between anthropometric parameters and visual acuity in the right eyes.

|          | Unstandardized Coefficients |            | Standardized Coefficients |       | Sig.    | 95.0% Confidence Interval for B |             | F      | P       |
|----------|-----------------------------|------------|---------------------------|-------|---------|---------------------------------|-------------|--------|---------|
|          | B                           | Std. Error | Beta                      | t     |         | Lower Bound                     | Upper Bound |        |         |
| Constant | 0.533                       | 0.119      |                           | 4.479 | 0.00001 | 0.299                           | 0.767       | 13.291 | 0.00032 |
| H        | 0.257                       | 0.071      | 0.180                     | 3.646 | 0.00032 | 0.118                           | 0.396       |        |         |

H = height; BMI = body mass index, B = Coefficient of regression; std error = standard error

The stepwise logistic regression evaluation for the association between the anthropometric parameters and the measurement of visual acuity in the left eyes failed to be generated by the SPSS software in the current study. This suggested that none of the anthropometric parameters was a good predictor of the left eye visual acuity in the current study.

### Discussion

In the current study, most of the study participants were males, had normal VA as well as BMI that was categorized as normal. The distribution of the HC, height and weight anthropometric measures of the study participants were significantly different when evaluated based on gender. The absolute proportion of participants that were obese and had abnormal VA was significantly higher than the number of participants that were obese and had normal VA regardless of the eye evaluated (right or left). The measurement of height was also significantly different when evaluated based on the type of VA (normal or abnormal) that was identified in the right and/or left eyes of the study participants. A linear regression model for predicting the VA in the right eye and not the left eye was identified. The model consisted of height as well as intercept value (constant) as predictive variables and it appears to be relatively robust based on the F test evaluation.

Height has been evaluated in the literature for an association with the level of refraction and the assessment suggested that when evaluating the entire study participants, there was no correlation between the evaluated parameter, but it was significant when the evaluations was limited to the female participants in the study. [1, 16, 19] Thus, suggesting that the eye has a capacity to adjust for axial length differences viz-a-viz VA levels in relation to height. [19]. These literature reports were not in keeping with

the finding in the current study as well as some other evaluations that had reported an association between height and VA. The value of reestablishing an association between VA and height as in this study suggests that the association between height and VA appears to be robust, however, the contrasting results that had been reported earlier in the literature remains a weak point for this conclusion. The reason for the difference in findings lies partly in the fact that some of the studies that reported findings that were not similar to those of the current study evaluated for refraction using an autorefractor after attaining a pupil diameter of  $\geq 6$  mm dimension and not just VA using Snellen's chart as done in the current study. [16, 19] One of the earlier studies that failed to establish an association between VA assessed using Snellen's chart with height may probably be because the study population were of the underweight BMI category while the current study had participants that were mainly categorized as belonging to the normal weight BMI category. [1] Another probable reason for the difference in association findings that have been suggested was the role of other factors that affect height and VA status independent of each of them. It has been postulated that the relationship between factors such as social status including the level of education as well as an economic status which contributes to the type of diets may have stronger influence beyond the direct relationship between height and refraction. [19] In addition, the regression model for the prediction of the VA of the right eye from height in this study is also a novel finding

To the best of our search, there are no works that documented an evaluation of an association between VA and HC. Thus, the assessment of a potential association between HC and VA as in the current study is a novel evaluation and thus could bolster the

goals for identifying appropriate anthropometric features that could predict VA. Nevertheless, the observation in the current study that there was no association between HC and VA remains a novel finding and it suggests that the size of the head viz-a-viz its shape may not have any measurable effect on VA, although this specific evaluation (evaluating the shape of the head) was not done in the current study.

An earlier study that evaluated the association between anthropometric parameters and VA which was populated by a large number of underweight study participants (mean BMI =  $16.6 \pm 3.22 \text{ kg/m}^2$ ) reported that there was a significant association between overweight BMI with abnormal VA. [35,36] Other studies that were characterized with a high prevalence of overweight/obesity children also reported that overweight and/obesity BMI was associated with poor VA scores. [37 – 39] There were other studies that reported an association between abnormal VA with the obese BMI category among children and young adults. [6, 35, 40] The current study was mainly populated by participants that were of the normal weight BMI category (mean BMI =  $20.97 \pm 3.48 \text{ kg/m}^2$ ) and had distinct overweight and obese categories of BMI which are key departures from some of the earlier literature report. Thus, these key differences in the BMI category of study participants may underlie the difference in the association finding of the VA with obese BMI observed in the current study unlike with the overweight category earlier reported in the literature. The current study as well as reports of association from the literature suggest that increasing BMI levels was associated with abnormal VA in the eyes. The actual mechanism involved may putatively be linked to the positive role played by increased BMI in the predisposition of individuals to ocular pathologies like diabetic retinopathy. [16, 19] These aforementioned mechanisms will hold true if the cause-effect relationship is from obesity to the abnormal VA which is not measurable in a cross-sectional study. Another mechanism that had been proposed entails the presumption that abnormalities in VA made the affected participants to drastically reduce their participation in sporting activities, increase their sedentary life style and thus become overweight/obese. [39,41,42] Indulging in TV watching and activities that are associated with looking at computer screens for extended periods are features

of a sedentary lifestyle and they are accompanied by excessive intake of calories and little outdoor activities which predispose to large BMI scores. [37, 43]

### 5.3 Conclusion

In conclusion, the distribution of height anthropometric measures was significantly different based on the type of VA in the right and left eye. Abnormal VA was associated with shorter heights in each eye. The study also shows that underweight, overweight and obese study participants recorded the highest risk for visual impairment compared to normal-weight participants. A predictive model for the determination of VA in the right eye was also established in the current study.

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## Sero-prevalence of Hepatitis B virus (HBV) infection among HIV seropositive and Tuberculosis Patients attending General Hospital Uba, Borno State, Nigeria

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### Abstract

The study determined sero-prevalence of Hepatitis B virus (HBV) infection of HB virus antigen among Human immunodeficiency virus (HIV) positive and Tuberculosis (TB) patients attending General Hospital Uba. The study was a cross-sectional study in which 461 patients were screened for HIV using HIV 1/2 STAT-PAK® Assay and tuberculosis using Mantoux tuberculin skin test (TST). The presence of HBV antigen among the patients was determined using Uni-Gold™ HBsAg. The results of the study showed that out of 461 patients studied 300 (65.08%) had HIV infection and 161 (34.92%) had tuberculosis. The prevalence of HBV among HIV patients was (30.00%) and the prevalence of HBV among the TB patients was (11.80%). Findings of the study showed that patients in the age group 30-39 years have the highest prevalence rate of 8.67 % of the HBV among HIV patients co-infected with HBV while age group 20-29 years has the highest prevalence rates of 16.33% among female patients co-infected with HIV and 16.21% among female patients co-infected with TB respectively. The study highlights the need for medical practitioners to lay emphasis on continuous screening of HIV and TB patients for HBV co-infection for early diagnosis and possible treatment.

**Keywords:** Hepatitis B Virus antigen, Sero-prevalence, HIV, TB, Co-infection

### Introduction

Hepatitis B virus (HBV) infection is a very serious health problem. Globally, it has been recorded that approximately 2 billion people are infected with HBV and 240 million people are chronically infected, with Africa and Asia having the highest number [1, 2]. Report by the World Health Organization (WHO) [3] showed that about 650,000 people die annually due to chronic HBV infection. The main complications of chronic HBV are attributed to cirrhosis and hepatocellular carcinoma.

HBV is predominantly spread through mucosal contact to infected blood and other body fluid such as saliva, seminal fluids, menstrual and vaginal fluid [4]. The virus may be transmitted through accidental inoculation of minute amounts of blood or fluid during medical and surgical procedures and from similar items contaminated with infected blood; use of unsterilized syringes and needles, intravenous and

percutaneous drug abuse, body piercing, tattooing and acupuncture [3].

The record showed that 4 million people infected with HBV are co-infected with HIV worldwide and most infected in Sub-Saharan Africans (SSA) with an expected high prevalence of co-infection [5, 6]. These infections co-exist due to their similarities in the mechanism of transmission and risk factors. Studies have shown that people co-infected with HBV and HIV have a high risk of developing chronic HBV infection which is ten times higher than that of the general population [7, 8, 9].

Tuberculosis (TB) and HBV are two major global health problems. The World Health Organization (WHO) [10] documented that approximately 2 billion people, that is about one-third of the world's population are infected with the causative agent of TB '*Mycobacterium tuberculosis*'. Record showed that in 2015 alone, approximately 10.4 million

newer cases of TB were recorded [10] while in 2018 it decreased to 10.0 million [11] and an estimated death of 1.67 million occurred in 2017 [12]. Among the reported death cases, 8.6 % were people living with HIV (PLHIV) [11].

Treatment of TB patients co-infected with HBV is a challenging health problem globally. Presently, rifampin (RFP), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) are drugs used for patients with drug-sensitive tuberculosis infection in their initial intensive anti-TB treatment as first-line treatment. Hepatotoxicity has been associated with these medications with an occurrence of 3 to 28 % idiosyncratic and intrinsic toxic reactions [13-16]. HBV is a non-cellular pathogen; however active HBV replication can lead to immune liver injury and even liver failure, especially in adults [17]. Hepatotoxicity of anti-TB medications and immune liver injury caused by HBV replication exacerbate liver dysfunction [18, 19]. It is against this background that this study seeks to determine the prevalence of co-infection of HBV among HIV and TB patients attending General Hospital Uba, Borno, State, Nigeria.

## **Methods**

### **Study Design**

This study was conducted among Persons Living with HIV and TB patients attending General Hospital Uba, Askira/Uba, Local Government Area, Borno State, Nigeria from February to July 2018.

### **Study Area**

The study was conducted at General Hospital Uba, Askira/Uba Local Government Area, Borno State, Nigeria. Askira/Uba Local Government Area extends from Samuwa in Lassa and located on Latitude: 10° 27' 18.32" N and Longitude: 13° 13' 20.39" E.

### **Study Population**

A total of 461 infected patients (males and females) of different age group (9 – 79 years) that reported to the general Hospital Uba were examined. HBV among HIV and TB patients were used. The patients' information and blood samples were collected and were assured of confidential use of data for research purposes only.

### **Sample Collection and Laboratory Analysis**

Two millilitres (2 ml) of blood samples were collected from each of infected patients into a sterile bottle containing 2 drops of EDTA for laboratory analysis. Presence of HIV was determined using HIV 1/2 STAT-PAK® Assay (Chembio Diagnostic Systems, Inc, NY, USA) [20] as described by the manufacturers, tuberculosis using Mantoux tuberculin skin test (TST) [21], with a tuberculin syringe, 0.1 mL of tuberculin purified protein derivative (PPD) was injected intradermally into the surface of each patient forearm with needle bevel facing the upward. The reactions were read on the skin between 2 to 3 days for induration (positive cases). The qualitative detection of HBsAg was conducted on whole blood using Uni-Gold™ HBsAg (Trinity Biotech Plc, Ireland) as described by the manufacturers.

### **Data Analysis**

Data was analyzed using descriptive statistics in SPSS version 24.

## **Results and Discussion**

A total of 461 patients that attended General Hospital Uba Local Government Area Borno State from February to July, 2018 were screened for the prevalence of Hepatitis B virus surface antigen (HBVsAg). The patients screened were in two categories. The first group consist of 300 patients who were HIV positive and screened for the prevalence of HBsAg while group two consist of 161 TB patients who were screened for the presence of HBsAg. The study was conducted to determine the presence of HBsAg in the patients based on age group and gender.

Firstly, the patients were screened for the prevalence of HBsAg among HIV infected patients. Co-infection of HBV with HIV is common due to their similar route of infection [22]. The results on basis of the age-related prevalence of HBsAg among HIV infected patients (Figure 1) revealed that age group 30-39 years has the highest prevalence rate of 8.67 % compared to other age groups and patients not infected with HBsAg. The results further revealed that age group 10-19 years and 20-29 years has an equivalent prevalence rate of 6.67 % while the lowest prevalence rate was recorded among age group 60-69 years of the 300 HIV patients examined. HIV patients not infected with HBsAg have the

lowest prevalence rate compared to HBsAg infected patients.

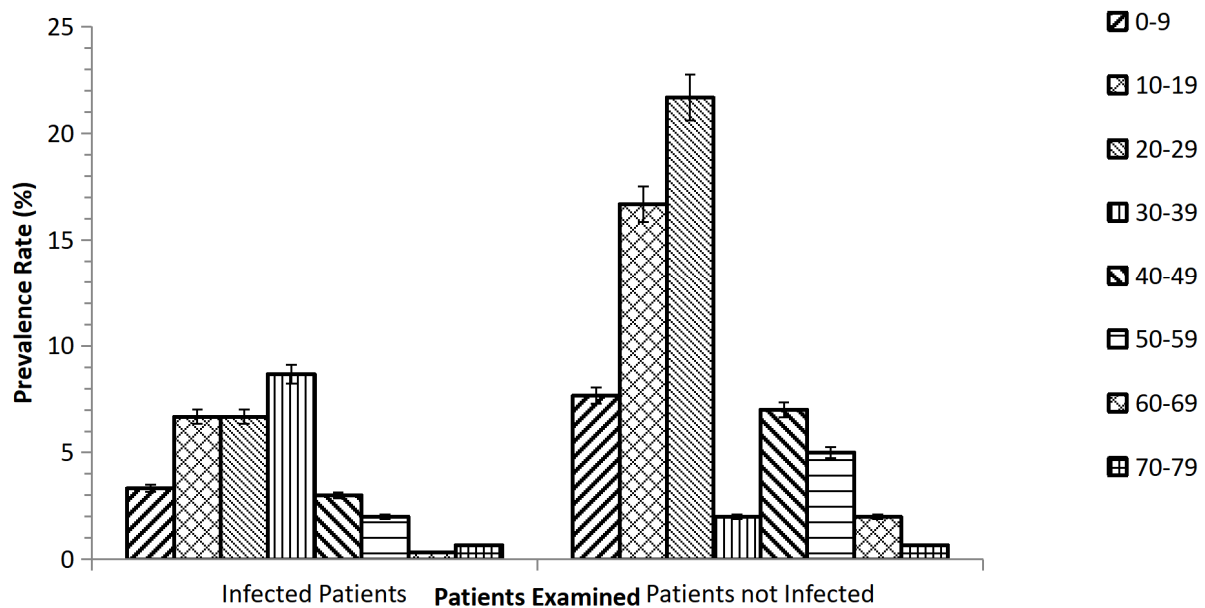
Studies on the prevalence rate of HBsAg infected TB patients as shown in Figure 2. The data obtained for 161 patients on the prevalence rate of HBsAg infected TB patients among different age groups revealed that 19 patients were positive for HBsAg while 142 patients were negative. Out of the patients screened, it was found that age groups 20-29 years having the highest prevalence rate (3.73 %) while age group 60-69 years had the lowest prevalence rate 0.62 %. Other prevalence rates were found distributed equally among the different age group. The result also showed that there was no HBsAg infected TB patients in the age group 50-59 and 70-79 years.

In addition, the result in Figure 3 showed gender-related prevalence of HBsAg among screened HIV patients. A total of 300 patients were screened (170 males and 130 females). It was found that 90 patients infected with HBsAg among HIV patients have a prevalence rate of 16.33 % among female patients and 13.67 % among male patients. This result showed that female patients have a higher prevalence rate compared to the male counterpart patients. The result also showed that 43 % and 27 % of male and female patients respectively were HIV positive had no cases of HBsAg infection. This study is in agreement with Taura *et al.* [20] for higher prevalence rate (9.1 %) of co-infection of HBV and TB among females than males. The present study also showed a higher prevalence rate (16.33 %) compared to their report. This could be attributed to the difference in the sample size used in

the study. They also showed a higher prevalence rate (4.2%) among age bracket 21-39 years which agreed with findings in this study for prevalence rate (3.73 %) among 20-29 years age bracket. However, the present report is not in line with the report that prevalence of HBsAg positivity decreases with age although relatively high in all age groups but higher in males (8.7%) who HIV infected than females (4.7%) of the same status [23].

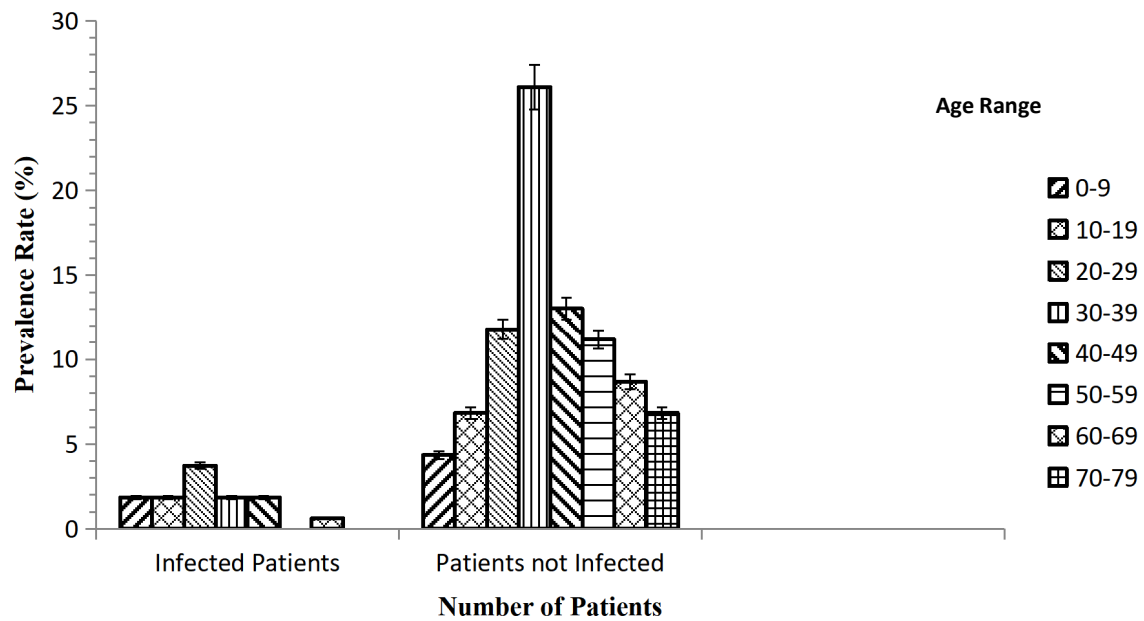
The present study on gender-specific prevalence rate of HBsAg infection among TB patients (Figure 4) among 161 TB patients screened, showed that female TB patients infected with HBsAg had a prevalence rate of 6.21 % against their male counterparts (3.11 %). The study further revealed that a total of 15 TB patients were infected with HBsAg while 146 TB patients were uninfected containing the highest number of patients. This observation contradicts the findings of Hamza [24] for the higher prevalence rate in male subjects for HBsAg co-infection HIV in a tertiary hospital in Nigeria.

The study agrees with the report by Jamda *et al.* [25] for higher prevalence among female HIV co-infected with TB attending secondary health centre in Abuja Nigeria. Similarly, in an attempt to determine the gender differences among subjects attending TB unit in Teaching Hospital in Southern Nigeria, [26] reported higher prevalence rate 51.2 % among female patients with HIV co-infected with TB compared to their males having 37 %.

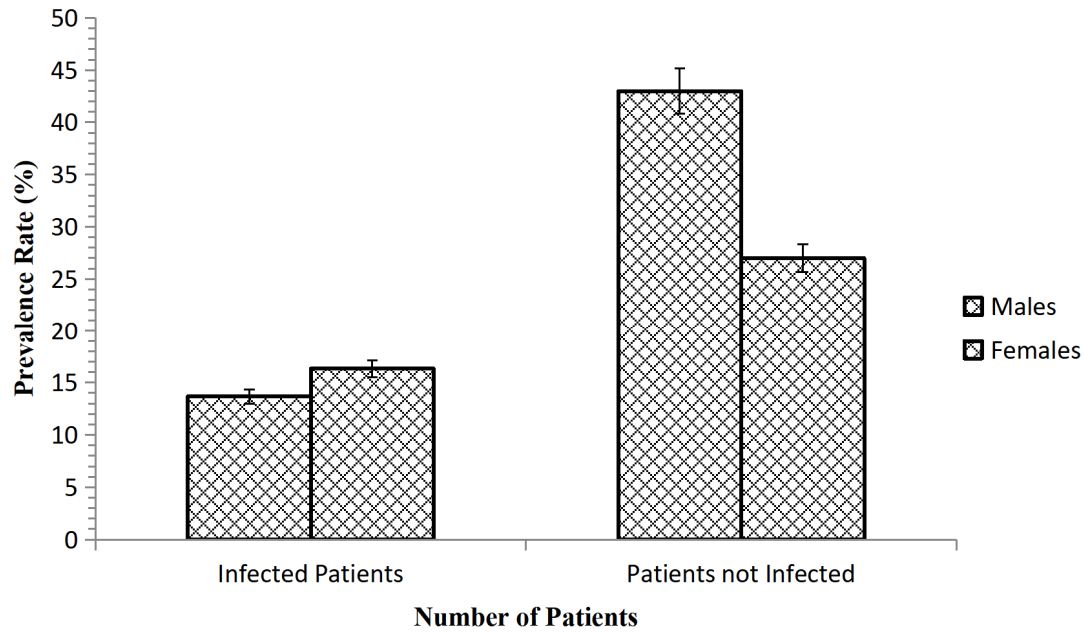


c

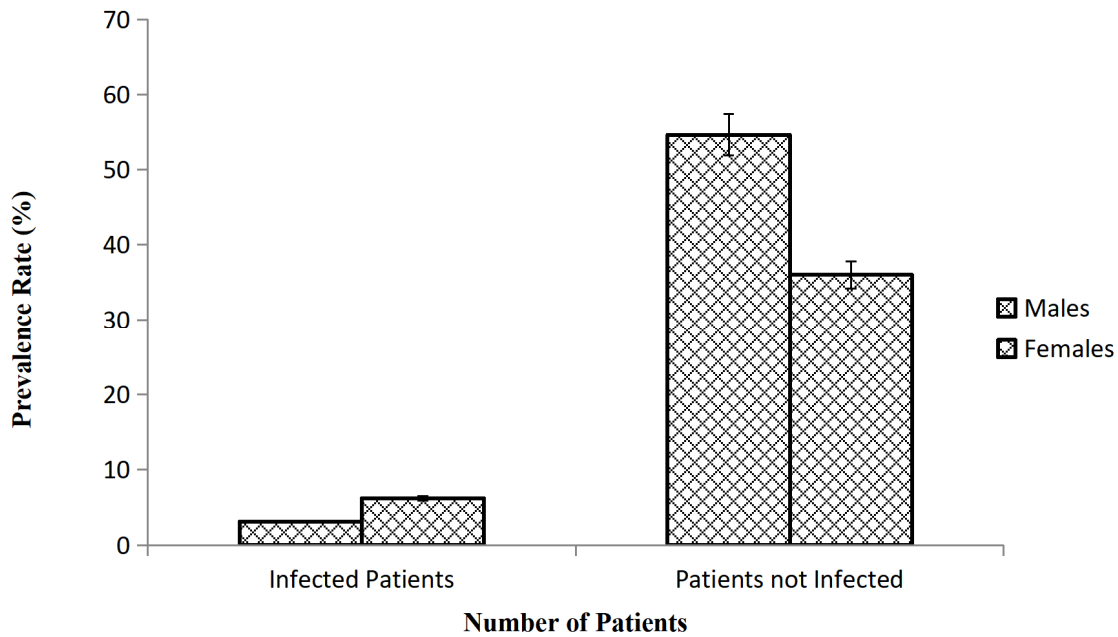
**Figure 1:** Age related prevalence of HBsAg in HIV Patients



**Figure 2:** Age related prevalence of HBsAg in TB patients



**Figure 3:** Gender-related prevalence of HBsAg in HIV patients



**Figure 4:** Gender-related prevalence of HBsAg among TB patients

## Conclusion

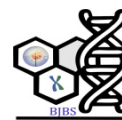
The findings from this study revealed the prevalence rate of Hepatitis B surface antigen in HIV and TB patients attending General Hospital Uba for six months. The study revealed the prevalence rate by age group and gender-specific. The study revealed that out of the 300 HIV and 161 TB patients among attending General Hospital Uba within the age group 30-39 years has the highest prevalence rate of 8.67 % among HBsAg infected HIV patients while the age group 20-29 years has the prevalence rate (3.73 %) among HBsAg infected TB patients. The study also revealed that female patients had the highest prevalence rate among HBsAg infected HIV and TB patients against their male counterparts. The findings in this study call for medical intervention among HIV and TB patients in order to further prevent the epidemic of the disease.

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## Spores and Metabolites of *Aspergillus niger* as Potential Biolarvicide of *Anopheles* Mosquito Larvae from North West Nigeria

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### Abstract

Biological control potential of *Aspergillus niger* against *Anopheles gambiae coluzzi* was evaluated. *A. niger* was isolated from soil using a soil suspension method with selective isolation media. The virulence of *A. niger* spores was tested against 4<sup>th</sup> instar larvae of *Anopheles gambiae coluzzi* using five different conidial concentrations;  $2.2 \times 10^6$ ,  $2.2 \times 10^5$ ,  $2.2 \times 10^4$ ,  $2.2 \times 10^3$  conidia/ml. The larval mortalities were observed for 48 hours. The mortality values were then subjected to probit analysis and LC<sub>50</sub> and LC<sub>90</sub> values were calculated. The percentage mortality of the mosquito larvae treated with different conidial concentration varied from 10 to 73%. The results revealed 73% as the maximum percentage mortality at the highest applied dose of  $2.2 \times 10^6$  conidia/ml and 27% for the lowest applied dose of  $2.2 \times 10^3$  conidia/ml. No mortality was observed in the control. Both LC<sub>50</sub> and LC<sub>90</sub> values after 24 hours were higher than those of 48 hours. Results of metabolites bioassay show that mortality in the positive controls (Insecticides; Temephos and Malathion) was 100% while that of a negative control (DMSO) was zero. For the tests, 40% was observed as the maximum percentage mortality at the highest applied dose of 500µg/ml of ethyl acetate extract whereas it was 13.3 and 30% for ethanol and diethyl ether extracts respectively. At higher concentrations of the most active extract, ethyl acetate extract, the percentage mortality increased to 100% at the highest applied concentration of 1000µg/ml. Metabolites from the most active extract were characterized using GC-MS and the results indicated the presence of the following compounds: penta-2-ol, 4-allyloxy-2-methyl, cyclopentanol, 1-methyl-11,14-octadecadinoic acid methyl ester and 4,8,12,16-tetramethylheptadecan-4-olide. These results indicate that *Aspergillus niger* has the potential to be a source of biological control agent for immature stages of *Anopheles* mosquitoes and should be exploited further to completely elucidate the active compounds and mechanisms of action.

**Keywords:** *Aspergillus niger*, Metabolites, Spores Mosquito Larvae, Biolarvicide

### Introduction

Malaria is a major disease of humans caused by protozoan parasites from the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. Five species of *Plasmodium* parasite cause malaria. [1] Infection by all *Plasmodium* spp. begins with the bite of an infected female *Anopheles* mosquito. [2] The first symptoms of malaria are nonspecific and similar to the symptoms of a minor systemic viral illness. Malaria is, therefore, frequently over-diagnosed based on symptoms alone, especially in endemic areas like Nigeria, because of this non-specificity of symptomatology. [3] Entomopathogenic

fungi (EPF) are a very heterogeneous group of fungi belonging to different systematic groups and with different biological characteristics. However, all of them are pathogenic in relation to insects and all arthropods, and their effectiveness in infecting their hosts is so large that it can become a factor regulating the abundance of insects. [4] The advantages of entomopathogenic fungi over synthetic insecticides are their significantly higher host specificity, reduction of environmental hazards and the inability of the insects to develop resistance; because the EPFs simultaneously use several modes of actions and as living-pesticides, they are also

subjected to adaptation. The slow efficacy in comparison to chemical pesticides and on-field exposure to various biotic and abiotic stresses is the main constraints to EPF. The EPF are unique comparatively and cause infection by growing through insect cuticle, therefore infectivity is caused by contact. This offers great potential in pest management.<sup>[5]</sup>

*Aspergillus niger* is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans.<sup>[22]</sup> *A. niger* is most widely known for its role as a citric acid producer.<sup>[23]</sup> With the production of citric acid at over one million metric tons annually, *A. niger* citric acid production serves as a model fungal fermentation process. This organism is a soil saprobe with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose. A variety of these enzymes from *A. niger* is important in the biotechnology industry. The *A. niger* is also an important model organism for several important research areas including the study of eukaryotic protein secretion in general, the effects of various environmental factors on suppressing or triggering the export of various biomass-degrading enzymes, molecular mechanisms critical to fermentation process development, and mechanisms involved in the control of fungal morphology. These are encouraging characteristics which encourage for further research on mosquitoes control. The objective of this study therefore is to evaluate the potential of *Aspergillus niger* spores and metabolites as a biocontrol agent for An. Mosquitoes.

## Materials and Methods

### Isolation and Identification of Fungi from Soil

The study was conducted at Bayero University Kano at 11°58'50"N 8°28'46"E. Soil samples were collected from insect hibernation sites including fields characterized by soil with a lot of leaf litters that cover the area. Exactly 100g each of soil sample were taken from four (4) different locations from a depth of 0 to 20cm with a trowel after removing litter and placed in appropriately labelled plastic bags. The entomopathogenic fungi were isolated from the soil using the soil dilution plating method.<sup>[6]</sup> Each soil sample (1g) was weighed and added separately to 9ml of sterile 0.01% (w/v) Tween-80 in a test tube and agitated. One hundred microliters

(100µl) of soil suspension were then placed aseptically above solidified PDA media in Petri dishes. The suspension was then spread using an L-shaped glass rod. The plates were prepared in triplicates and incubated at room temperature until the growth of fungi was observed.

After three (3) to five (5) days, fungal growths of different colonies were observed in the plates. A strand of mycelium was then aseptically transferred onto selective PDA media containing cetyl trimethyl ammonium bromide (CTAB) and chloramphenicol and incubated in the dark for about five (5) days to obtain pure colonies. Pure fungal growth was observed after five (5) days.

To identify entomopathogenic fungi from the soil, pure colonies of the fungi were prepared on microscope slides. A sterile needle was used to collect a strand of mycelia and placed on a glass slide to study for micro-morphological features using a microscope (Olympus CX31 series, England) with magnification X10 and X100. The macroscopic and microscopic characteristics of the isolated fungi were examined using reference text.<sup>[7]</sup>

### Production of Conidial Suspension and Counting of Spores

A mixture of conidia and hyphae was harvested by flooding the Petri dishes with 10 ml of sterile distilled water in 0.05% Tween 80 (Sigma, USA). The resultant suspension was then poured into a test tube and vortexed to produce a homogenous suspension. The concentration of the conidia in the solution was determined by direct counting using a hemocytometer (Bright Line, Sigma-Aldrich, Germany)<sup>[8]</sup> under a compound microscope. After getting an established concentration of  $2.2 \times 10^6$  conidia/ml, the suspension was diluted with 0.01% Tween-80 to the concentrations of  $2.2 \times 10^5$ ,  $2.2 \times 10^4$  and  $2.2 \times 10^3$  conidia/ml.

### Preparation of Extract

Preparation of extract was carried out using methods described by Perumal *et al.*<sup>[9]</sup> Fungal spores were harvested by flooding sterile distilled water with 0.05% Tween 80. The fungal suspension was then filtered using a sterile cloth to remove fungal hyphae, conidia clumps and media debris. The spore concentration in the filtered fungal suspension was estimated using hemocytometer to count the spore

concentration and then adjusted to  $2.2 \times 10^6$  conidia/ml. This was then inoculated in 250 ml of potato dextrose broth (PDB), and incubated at 28°C and 130rpm in an orbital shaker. After seven (7) days, the fungal biomass was filtered through a muslin cloth. Then, the biomass was washed with sterile distilled water three (3) times for the removal of media components. A quantity (5 g) of fungal biomass was transferred to three (3) different conical flasks containing 250 ml of ethyl acetate, ethanol and diethyl ether respectively and extracted for seven (7) days using an orbital shaker at 130 rpm and 28°C. After incubation, the fungal filtrate was filtered through a muslin cloth. The fungal crude filtrate was further concentrated to eliminate ethyl acetate, ethanol and diethyl ether by using a rotary evaporator under reduced pressure 25 mmHg at 45°C and the residue obtained was stored at room temperature.

### Mosquito Sampling and Larvae Rearing

Third and early fourth instars *Anopheles* mosquito larvae were sampled from stagnant breeding water in Auyo local government area of Jigawa State and brought in and maintained in the laboratory at a temperature of 31°C and relative humidity of 722.9mmHG. After conducting bioassay, 30 larvae were randomly selected and subjected to species identification using *Anopheles gambiae* specific SINE 200 primers

### Larvicidal Bioassay

Laboratory bioassay was carried out using standard methods [10] with slight modifications. Fungal conidia were tested against 3<sup>rd</sup> and early 4<sup>th</sup> instar larvae of *Anopheles* mosquito by adding the fungal suspension to a plastic disposable cup containing 20ml of distilled water with 10 larvae. Each cup was inoculated with 1ml of each fungal suspension ( $2.2 \times 10^6$ ,  $2.2 \times 10^5$ ,  $2.2 \times 10^4$  and  $2.2 \times 10^3$  conidia/ml). Control treatments were carried out by adding 20ml of distilled water only. Each concentration was replicated three times. Larval mortality was observed at 24 and 48 hours and recorded.

Bioassay of the fungal extracts was also carried out following the methods of Bensarady and Mihoubi [10] with slight alterations. Each of the dried extracts was weighed and dissolved in DMSO to form a solution/suspension. Each fungal extract was tested

against 4<sup>th</sup> instar larvae of *Anopheles* mosquito larvae by adding the fungal suspension to a plastic disposable cup containing ten (10) larvae with 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml of extract and filled up to 20ml with distilled water. Each concentration of the different extracts was replicated three times. Control treatments were carried out by adding 1ml each of DMSO (negative control), and chemical insecticides, malathion and temephos (positive control). Larval mortality was observed and recorded after 24 hours. The most active extract (with highest larval mortality) was tested against 4<sup>th</sup> instar larvae of *Anopheles* mosquito larvae again using the same setup but higher concentrations (600, 700, 800, 900 and 1000µg/ml). Larval mortality was also observed and recorded after 24 hours.

### Data Analysis

The data obtained were subjected to probit analysis. [11] The relationships between probit and log concentrations were established as probit equations and probit regression lines were drawn. Lethal concentration causing 50% and 90% mortality (LC<sub>50</sub> and LC<sub>90</sub> respectively) were also estimated from the probit equation.

### Extract Characterization

The dried residue of the most active extract was dissolved in n-hexane and then filtered through a 0.2µm syringe filter before ingestion into a GC-MS column (Agilent DB-5MS column, 30 m×0.25 mm I.D., 0.25 µm film thickness) using helium as carrier gas at 1ml/min flow rate. The oven temperature was programmed at 60°C initial temperature for 2 mins, which then rises at a rate of 10°Cmin<sup>-1</sup> to 300°C and finally held isothermally at 300°C for 6mins, to complete a total run time of 34mins. Bioactive compounds were identified by comparing their mass spectra and retention indices with those of NIST mass spectral library.

### Results and Discussion

#### Bioassay

The present study evaluated larvicidal properties of *Aspergillus niger* conidial suspension against *Anopheles gambiae coluzzi* larvae. The virulence of *A. niger* against the *An. gambiae coluzzi* larvae resulted in a significant mortality of the larvae reaching 73% (Table 1). The percentage mortality of

the larvae increased with increasing concentration and time. This result also correlates with previous studies [12] [13] [10] [14][15] which prove that fungal isolates take time to kill different mosquito species depending upon the dose and the type of fungal strain. The use of fungal spores as a larvicide could

complement adult control but only in areas where the breeding site are well defined and are not being used for domestic purposes. [16] The efficacy of conidial suspension of *Aspergillus niger* on the 4<sup>th</sup> instars larvae was also expressed in terms of LC<sub>50</sub> and LC<sub>90</sub> (Table 1).

**Table 1:** Larvicidal Efficacy of *A. niger* Conidial Suspension Against *Anopheles* Mosquito Larvae.

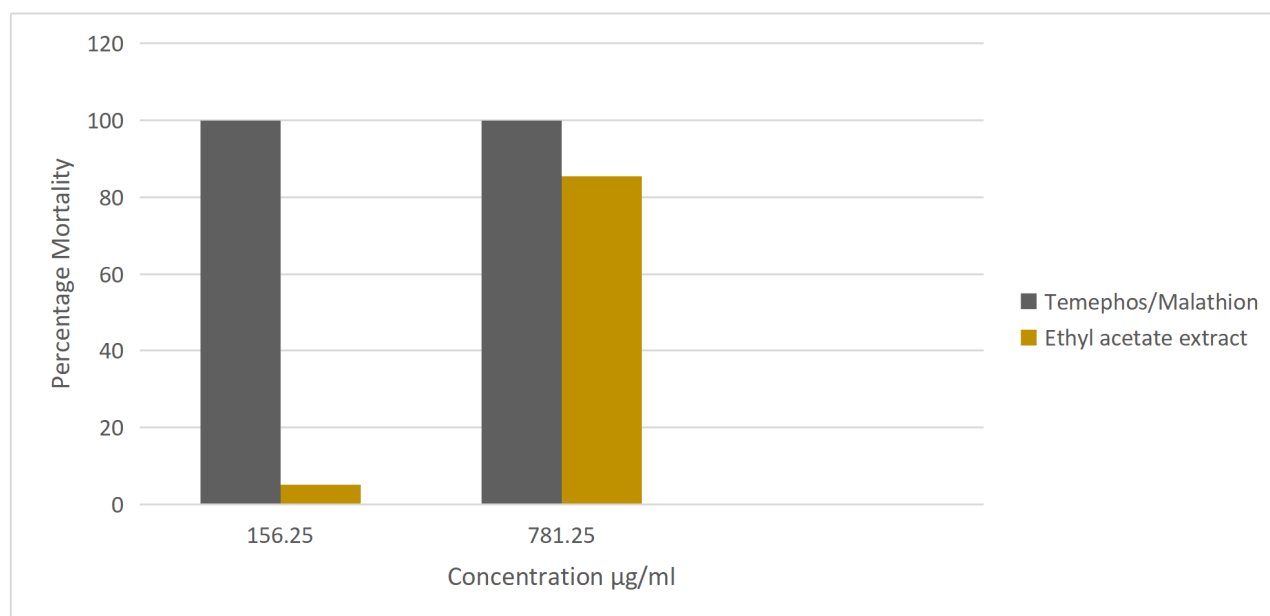
| Exposure time | Concentration (conidia/ml) | Percentage Mortality | LC <sub>50</sub> (conidia/ml) | LC <sub>90</sub> (conidia/ml) |
|---------------|----------------------------|----------------------|-------------------------------|-------------------------------|
| 24hours       | 2.2×10 <sup>6</sup>        | 30                   | 1.4x10 <sup>8</sup>           | 4.7x10 <sup>4</sup>           |
|               | 2.2×10 <sup>5</sup>        | 27                   |                               |                               |
|               | 2.2 x 10 <sup>4</sup>      | 17                   |                               |                               |
|               | 2.2 x 10 <sup>3</sup>      | 10                   |                               |                               |
| 48hours       | 2.2 x 10 <sup>6</sup>      | 73                   | 1.3x10 <sup>13</sup>          | 3.5x10 <sup>7</sup>           |
|               | 2.2 x 10 <sup>5</sup>      | 67                   |                               |                               |
|               | 2.2 x 10 <sup>4</sup>      | 43                   |                               |                               |
|               | 2.2 x 10 <sup>3</sup>      | 27                   |                               |                               |

In the present study, metabolites extracted from *A. niger* using three different solvents were found to be effective against 4<sup>th</sup> instar larvae of *Anopheles* mosquito. Pathogenicity however varied according to the concentration of extracts and the type of solvent used for the extraction. Of the three solvents, extracts of ethyl acetate had the highest pathogenic effect. Results revealed 40% as the maximum percentage mortality at the highest applied dosage of 500µg/ml of ethyl acetate extract. Whereas it was 13.3% and 30% for the highest applied concentration of 500µg/ml ethanol and diethyl ether extracts respectively (Figure 2). The entomopathogenic fungi

have the ability to directly infect the host insect by penetrating into the cuticle and do not require ingesting by the insect to cause diseases. Metabolites extracted from the fungi may destroy the cuticle layer of the larvae and, which leads to the death of the larvae. [17] A similar result obtained by previous work [17] showed the efficacy of *B. bassiana* metabolites against the larvae of *Anopheles*, *Culex* and *Aedes* species with 100% mortality. Extracts of ethyl acetate were further compared to standard chemical insecticides.

**Table 2:** Larvicidal Efficacy of *A. niger* Extracted Metabolites against *Anopheles* Mosquito Larvae.

| Extracts                     | Concentration (µg/ml) | Percentage Mortality | LC <sub>50</sub> | LC <sub>90</sub> |
|------------------------------|-----------------------|----------------------|------------------|------------------|
| Ethanol                      | 100                   | 0                    | 707.86           | 1249.04          |
|                              | 200                   | 3.33                 |                  |                  |
|                              | 300                   | 3.33                 |                  |                  |
|                              | 400                   | 6.67                 |                  |                  |
|                              | 500                   | 13.33                |                  |                  |
| Diethyl ether                | 100                   | 0                    | 574.41           | 857.09           |
|                              | 200                   | 6.67                 |                  |                  |
|                              | 300                   | 13.33                |                  |                  |
|                              | 400                   | 16.67                |                  |                  |
|                              | 500                   | 30                   |                  |                  |
| Ethyl acetate                | 100                   | 3.33                 | 506.59           | 732.95           |
|                              | 200                   | 6.67                 |                  |                  |
|                              | 300                   | 23.33                |                  |                  |
|                              | 400                   | 30                   |                  |                  |
|                              | 500                   | 40                   |                  |                  |
| DMSO (Negative Control)      | 100                   | 0                    |                  |                  |
| Temephos (Positive Control)  | 100                   | 100                  |                  |                  |
| Malathion (Positive Control) | 100                   | 100                  |                  |                  |

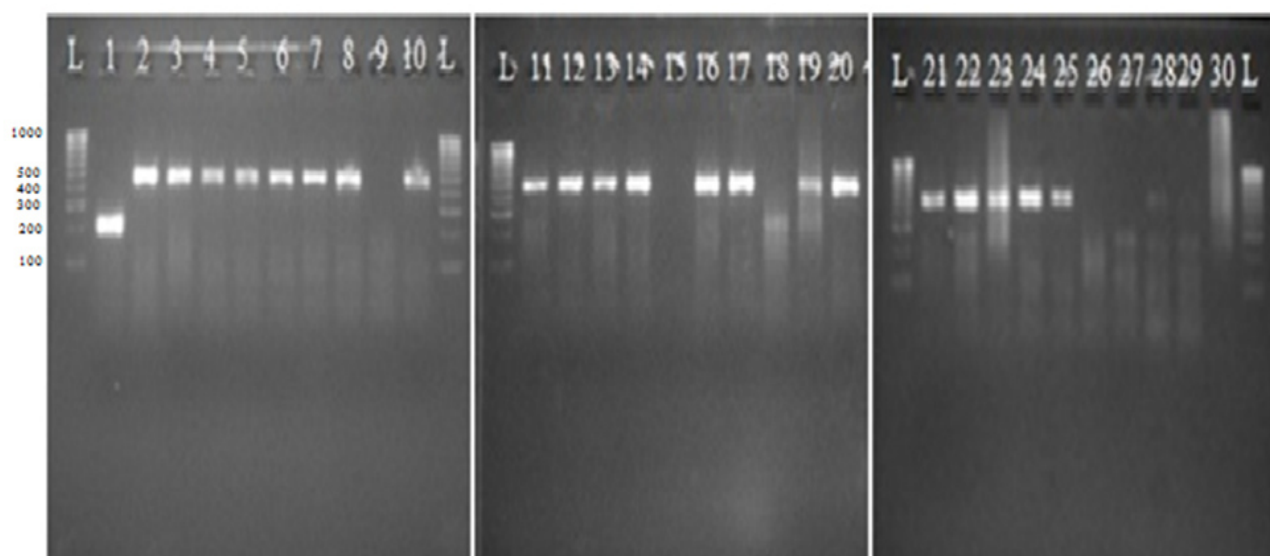


**Figure 1:** Comparison between larvicidal efficacy of ethyl acetate extracts of *Aspergillus niger* and standard chemical insecticides

### Anopheles Mosquito Species identification

Gel electrophoreses of SINE200 PCR products of the samples analyzed showed distinct bands corresponding to *Anopheles gambiae* S-Form and *Anopheles colluzzi* (Figure 2). Of the samples analyzed, 13% showed bands within 200bp, and are grouped as *Anopheles gambiae* S-Form, while 73% are around 500bp, and are grouped as *Anopheles colluzzi*, formally known as *Anopheles gambiae* m-form (80%).<sup>[21]</sup> The remaining failed reactions may be due to failed amplification or the larvae sampled did not belong to *Anopheles gambiae* (Sensu lato) group.

These two species of mosquito were previously reported to be abundant in Guinea and Sudan savannah region of northern Nigeria where sampling for this research was conducted. Also these two mosquito species along with others like *A. funestus*, *A. stephensi* and *An. arabiensis* are efficient vectors of malaria parasite; *Plasmodium falciparum*.<sup>[2]</sup> Most of these mosquito species have been reported to be resistant against many synthetic insecticides used in mosquito control.<sup>[2]</sup> Therefore their relative susceptibility to *A. niger* extracted metabolites and conidial suspension observed in this study could provide an alternative to the use of chemical insecticides.



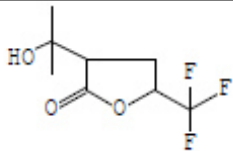
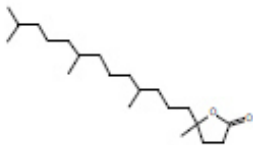
**Figure 2:** Agarose gel of SINE200 PCR Showing *Anopheles colluzzi* and *An. gambiae* S-form. Lane L- DNA ladder (1000bp), Lane 1, 18, 28 and 29- *An. gambiae* S-form (249bp), Lane 2-8, 10, 11-14, 16, 17, 20 and 21- *An. colluzzi* (479bp).

### 3.2 GC-MS Analysis of the Most Active Extract

Results of GC-MS analysis of ethyl acetate extracted metabolites from *Aspergillus niger* revealed a total of eleven (11) peaks corresponding to the presence of 11 compounds. The major constituents include tetrahydrofuran-2-one (5.448%), cyclopentanol, 1-methyl- (6.29%), 11,14-octadecanoic acid methyl ester (14.6%) and 4,8,12,16-tetramethylheptadecan-4-olide (22.24%) (Table 3). Notably, it has been earlier elucidated that carbonic acid bis(1-methylethyl) ester<sup>[18]</sup> and heptadecanoic acid, 16-methyl, methyl ester<sup>[19]</sup> possess insecticidal activity allowing us to argue that 11,14-octadecanoic acid

methyl ester found in the ethyl acetate extract of *Aspergillus niger* might be actively involved in the observed larvicidal effect in this work. A major advantage of using natural product metabolites as potential biolarvicides is that they limit the use of chemical insecticides thereby reducing environmental pollution. Natural metabolites also exert their toxicity through multiple mechanisms of action thereby reducing the possibility of development of insecticide resistance.<sup>[20]</sup> Fungal extract induces mechanical stress on the larvae and it also contains toxic compounds.

**Table 3:** Structural identity by mass spectrometry of compounds separated by gas chromatography.

| Peak | Retention Time | Area  | Molecular Weight | Name                                    | Structure                                                                           |
|------|----------------|-------|------------------|-----------------------------------------|-------------------------------------------------------------------------------------|
| 2    | 5.448          | 27211 | 212              | Tetrahydrofuran-2-one                   |  |
| 4    | 6.293          | 79811 | 100              | 1-methylcyclopentanol                   |  |
| 7    | 14.600         | 22490 | 290              | 11,14-Octadecanoic acid methyl ester    |  |
| 11   | 22.236         | 17311 | 324              | 4,8,12,16-Tetramethylheptadecan-4-olide |  |

## Conclusion

*Aspergillus niger* conidial suspension and extracts produced mortality in 4<sup>th</sup> instar larvae of *Anopheles gambiae colluzi*. Characterization of the extracts showed 11,14-octadecanoic acid methyl ester as one of the constituents of the metabolic extract of the fungi. This suggests that 11,14-octadecanoic acid methyl ester is probably the active principle responsible for the larvicidal action. These results indicate that *A. niger* has the potential to be a source of bio control agent for immature stages of *Anopheles gambiae coluzzi*.

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## The Evaluation of Variation in Skinfold Thickness During Growth among Nigerian School Children in Kaduna, Nigeria

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### Abstract

The development of skinfold measurements came as the result of investigations for simpler and less expensive methods for estimation of body composition including the regional distribution of body fat, occur during growth. This is important in the establishment of reference standards for body proportions, dimensions and also in clinical medicine for the assessment of genetic, environmental and nutritional status of children. This study aimed to determine variation in skinfold thickness measurements among Nigerians school children resident in Kaduna metropolis. A total of 1, 220 subjects (689 females and 531 males), recruited from public and private schools aged 8 - 18 years in Kaduna metropolis, were employed for this study. Anthropometric measurements of height, weight and skinfold thicknesses were taken using standard protocols. A student *t*-test was used to compare skinfold thicknesses in both sexes during growth. Sexual dimorphism index (SDI) was calculated using: Male mean/Female mean\*100. The data were subjected to statistical analysis of mean and standard deviation using student t-test and  $p \leq 0.05$  was considered statistically significant. Statistically, significant variation was observed in the skinfold thickness measurements: triceps (TSF), subscapular (SSF), supraspinale (SPSF) and medial calf (MCS) skinfolds of subjects. The females had higher values than the males at all developmental stages, with averages of males/females as: Age(years):  $13.78 \pm 2.65 / 13.74 \pm 2.61$ , TSF(mm):  $6.20 \pm 2.80 / 9.40 \pm 10.20$ , SSF(mm):  $6.90 \pm 7.20 / 9.10 \pm 8.10$ , SPSF(mm):  $6.10 \pm 2.90 / 11.10 \pm 5.00$ , MCS(mm):  $7.70 \pm 4.80 / 8.80 \pm 4.30$ . There is variation in skinfold thickness parameters of children with advancing age, with the females having more subcutaneous fat than males and this could be attributed to the preparation of the enormous energetic demands of reproduction in females.

**Keywords:** Anthropometry, Skinfold Thickness, Kaduna Metropolis, Sexual Dimorphism, Developmental Stage.

### Introduction

Skinfold measurement for body composition refers to the use of special callipers, simple to perform and low in cost to estimate body fat through the measurement of double, compressed thicknesses of subcutaneous fat and skin at various sites of the body [1]. It is important to note that excess adipose tissue, rather than excess weight in general, is the cause of the comorbid conditions [2]. This technique estimates body fat based on measurements of subcutaneous fat at various sites throughout the body, most commonly the triceps and subscapular regions [3]. Rolland-Cachera [4] demonstrated that one can reasonably predict total body fat from skinfold thickness at the triceps, and percent body fat from skinfold thickness at the subscapular site. Skinfold thickness has been shown to correlate well ( $r = 0.84$ -

0.93) with body fat, dependent upon the site measured and the sex of the subject [5]. Due to its direct measurement of subcutaneous body fat, skinfold thickness is more closely associated with body fat than Body Mass Index (BMI) [6].

Anthropometric measurements are not only important in the establishment of reference standards for body proportions and dimensions but also in clinical medicine and in the assessment of the genetic, environmental and nutritional status of children [7]. It is used by companies for proper and adequate production and management of goods and resources [7]. It also serves as biomarkers for disease conditions [8,9]. Body compositional changes, including the regional distribution of body fat, are especially large during the pubertal transition and

higher amounts of body fat and obesity are associated with increased risks of adverse health events and greater mortality <sup>[10]</sup>.

Skinfolds provide useful indicators of children and adolescents subcutaneous fatness and interbody study comparison can be based upon raw scores without attempting to convert to percentage body fat <sup>[11]</sup>. Children accumulate proportionally more subcutaneous fat on the extremities than on the trunk from infancy to about 5-6 years. Then, proportionally more subcutaneous fat accumulates gradually on the trunk until adolescence <sup>[11]</sup>. During the adolescent spurt, males gain more subcutaneous fat on the trunk, while fat on the extremities decreases. In contrast, girls gain proportionally equal amounts of subcutaneous fat on both trunk and extremities during adolescence. These observations are based on skinfolds measured on several trunk sites seen as, subscapular, suprailiac, abdominal and extremity sites seen as, triceps, biceps, medial calf <sup>[12]</sup>. The concept of skinfold measurement came up as a result of its simpler and less expensive nature and use of estimating body composition. They are termed generalized because they are most accurate in predicting body composition of people with average amounts of body fat. A person's body is made up of many different components, some of the major components are bone, muscle, organs and fat. Higher amounts of body fat and obesity are associated with increased risks of adverse health events and greater mortality <sup>[10]</sup>.

This study aimed at providing data to assess growth, sexual dimorphism in body fat distribution using skinfold thicknesses and for provision of reference data which will assists economic planning and health monitoring.

### **Materials and Methods**

The study was a cross-sectional survey of 1200 subjects (531 males and 689 females) within the age range of 8 to 18 years. The study sample consisted of school children from randomly selected public and private educational institutions (primary and secondary) in Kaduna North and Kaduna South local government areas in Kaduna metropolis conducted in the year 2013. Participating subjects were apparently healthy Nigerian children, without visible deformity. Classification based on developmental

stages was into Prepubertal age ranges from 8 to 10 years old, pubertal age ranges from 11 to 15 years old and posts pubertal age ranges from 16 to 18 years old <sup>[13]</sup>. Tools used for the study are weighing scale, anthropometer and the slim guide skinfold calliper. Ethical approval was obtained from the Kaduna State Ministry of Health research ethics committee.

### **Anthropometric Measurements of Weight And Height**

According to standard procedures reported by Lohman <sup>[14]</sup>; the body weight was measured to the nearest 0.5 kg with a portable weighing scale and subjects wearing minimal clothing and height was measured with a portable anthropometer with an accuracy of 0.1cm. Subjects were asked to inhale deeply and maintained fully erect position during the measurement. Body mass index (BMI) (kg/m<sup>2</sup>) was calculated from the subject's weight (body mass) relative to height, using the equation. BMI = weight (kg)/height (m<sup>2</sup>).

### **Anthropometric Measurements of Skinfold**

Measurement of skinfold thickness requires pinching a fold of skin and subcutaneous fat using the thumb and index finger, pulling it away from the muscle tissue, after determining the correct measurement site and then using the slim guide skinfold callipers and wait for 2 seconds before reading the callipers to measure the thickness. <sup>[15]</sup>

Fat is compressible, so reading the scale before or after the 2 seconds delay may affect the results. Independent measurements were taken by research technicians, resulting in two minimum observations for each side and the average recorded. The skinfolds measured include;

Triceps skinfold (TSF): subject's arm hanged loosely in anatomical position, a fold was raised at the back of the arm at a level halfway on a line connecting the acromion and the olecranon processes, Pinch the skin so that the fold is running vertically with the slim guide skinfold calliper to the nearest 0.05mm.

Subscapular skinfold (SSF): the skinfold was raised on a line from the inferior angle of the scapula in a direction that is obliquely downwards and laterally

at 45° and measurement taken with the slim guide skinfold calliper to the nearest 0.05mm.

measured with slim guide skinfold calliper to the nearest 0.05 mm.

Supraspinal skinfold (SPSF): the fold was taken above the anterior superior iliac spine on a line to the anterior axillary border and on a diagonal line going downwards and medially at 45 ° and skin thickness

Medial calf skinfold (MCS): a vertical fold was raised on the medial side of the leg, at the level of the maximum girth of the calf, and the skin pinched was measured with slim guide skinfold calliper to the nearest 0.05 mm.

## Results

Table 1: Descriptive statistics and skinfold thicknesses of subjects

| S/N | Parameters               | Male (N=531)<br>Mean±SD | Female (N=689)<br>Mean±SD | p-value |
|-----|--------------------------|-------------------------|---------------------------|---------|
| 1   | Age(years)               | 13.78±2.65              | 13.74±2.61                | 0.152   |
| 2   | Height(cm)               | 143.72±13.52            | 142.62±10.65              | 0.112   |
| 3   | Weight (kg)              | 42.93±10.63             | 43.92±10.65               | 0.104   |
| 4   | BMI (Kg/m <sup>2</sup> ) | 20.54±3.04              | 21.39±0.44                | 0.172   |
| 5   | TSF (mm)                 | 6.20±2.80               | 9.40±10.20                | 0.001   |
| 6   | SSF (mm)                 | 6.90±7.20               | 9.10±8.10                 | 0.001   |
| 7   | SPSF (mm)                | 6.10±2.90               | 11.10±5.00                | 0.001   |
| 8   | MCS (mm)                 | 7.70±4.80               | 8.80±4.30                 | 0.001   |

SD: standard deviation, n: number of subjects

Table 2: Sex variation in skinfold thicknesses of subjects (8 to 10 years)

| S/N | Parameters               | Male(n=77)<br>Mean±SD | Female(n=92)<br>Mean±SD | 95%CI  | t-value | p-value | SDI   |
|-----|--------------------------|-----------------------|-------------------------|--------|---------|---------|-------|
| 1   | Age (years)              | 9.39± 0.69            | 9.23 ± 0.74             | 0.161  | 1.451   | 0.149   | 101.7 |
| 2   | Height (cm)              | 127.27±7.37           | 130.58±9.94             | 3.308  | 2.416   | 0.017   | 97.5  |
| 3   | Weight (kg)              | 30.89±4.23            | 30.80±7.11              | -0.085 | 0.092   | 0.927   | 100.3 |
| 4   | BMI (kg/m <sup>2</sup> ) | 19.16±2.73            | 17.99±2.96              | -1.165 | 2.641   | 0.009   | 106.5 |
| 5   | TSF (cm)                 | 0.54±0.18             | 0.68±0.34               | 0.141  | 3.259   | 0.001   | 79.4  |
| 6   | SSF (cm)                 | 0.56±0.18             | 0.88±0.18               | 0.321  | 1.596   | 0.113   | 63.6  |
| 7   | SPSF (cm)                | 0.51±0.18             | 0.80±0.32               | 0.292  | 7.061   | 0.001   | 63.8  |
| 8   | MCS (cm)                 | 0.61±0.21             | 0.73±0.30               | 0.113  | 2.786   | 0.006   | 83.6  |

SD: standard deviation, n: number of subjects, SDI: sexual dimorphism index

Table 3: Sex Variation in Skinfold Thicknesses of Subjects (11 to 15 years)

| S/N | Parameters               | Male(n=290)<br>Mean±SD | Female(n=409)<br>Mean±SD | 95%CI | t-value | p-value | SDI  |
|-----|--------------------------|------------------------|--------------------------|-------|---------|---------|------|
| 1   | Age (years)              | 13.20±1.35             | 13.21±1.33               | 0.016 | 0.151   | 0.875   | 99.9 |
| 2   | Height (cm)              | 142.03±11.00           | 142.35±9.12              | 0.318 | 0.4173  | 0.677   | 99.7 |
| 3   | Weight (kg)              | 40.74±8.61             | 43.21±9.00               | 2.475 | 3.649   | 0.003   | 94.3 |
| 4   | BMI (kg/m <sup>2</sup> ) | 20.07±2.83             | 21.22±3.44               | 1.152 | 4.690   | 0.001   | 94.6 |
| 5   | TSF (cm)                 | 0.63±0.30              | 0.89±0.79                | 0.260 | 5.326   | 0.001   | 70.8 |
| 6   | SSF (cm)                 | 0.65±0.22              | 0.84±0.35                | 0.198 | 8.496   | 0.001   | 77.4 |
| 7   | SPSF (cm)                | 0.58±0.26              | 1.09±0.49                | 0.508 | 15.980  | 0.001   | 53.2 |
| 8   | MCS (cm)                 | 0.76±0.29              | 0.85±0.32                | 0.088 | 3.723   | 0.002   | 89.4 |

SD: standard deviation, n: number of subjects, SDI: sexual dimorphism index

**Table 4: Sex Variation in Skinfold Thicknesses of Subjects (16 To 18 Years)**

| S/N | Parameters               | Male(n=163)  | Female(n=189) | 95%CI  | t-value | P-value | SDI   |
|-----|--------------------------|--------------|---------------|--------|---------|---------|-------|
|     |                          | Mean±SD      | Mean±SD       |        |         |         |       |
| 1   | Age (years)              | 16.85±0.92   | 16.98±0.81    | 0.126  | 1.367   | 0.172   | 99.2  |
| 2   | Height (cm)              | 154.49±10.33 | 149.12±8.58   | -5.371 | 5.328   | 0.001   | 103.6 |
| 3   | Weight (kg)              | 52.50±7.71   | 51.83±6.97    | -0.675 | 0.8628  | 0.389   | 101.3 |
| 4   | BMI (kg/m <sup>2</sup> ) | 22.02±2.97   | 23.42±3.42    | 1.400  | 4.064   | 0.001   | 94.0  |
| 9   | TSF (cm)                 | 0.63±0.27    | 1.18±1.54     | 0.546  | 4.471   | 0.001   | 53.4  |
| 10  | SSF (cm)                 | 0.75±0.21    | 1.08±0.77     | 0.333  | 5.364   | 0.001   | 69.4  |
| 11  | SPSF (cm)                | 0.71±0.33    | 1.31±0.49     | 0.603  | 13.297  | 0.001   | 54.2  |
| 12  | MCS (cm)                 | 0.85±0.75    | 1.03±0.61     | 0.179  | 2.457   | 0.015   | 82.5  |

SD: standard deviation, n: number of subjects, SDI: sexual dimorphism index

Table 1: shows the mean skinfold thicknesses of all male and female school children aged 8 to 18 years, Significant difference was observed in TSF, SSF, SPSF and MCS, with the females having higher subcutaneous fat than their male counterparts.

Table II shows the mean skinfold thicknesses of male and female prepubertal school children aged 8 to 10 years, Significant difference was observed in TSF, SPSF and MCS, though no remarkable sexual dimorphism exists between them.

Table III shows the mean skinfold thicknesses of male and female pubertal school children aged 11 to 15 years. A significant difference was observed in TSF, SSF, SPSF, though no remarkable sexual dimorphism was observed.

Table IV shows the mean skinfold thickness of male and female post-pubertal school children aged 16 to 18 years. The significant difference was observed in TSF, SSF, SPSF and MCS.

The Sexual dimorphism Index (SDI) indicates the percentage difference between the sexes.

## Discussion

The findings from this research showed relatively small variation in anthropometric parameters between boys and girls as indicated by SDI, before puberty, with the girls having slightly more adipose tissue than the boys and this finding is in line with studies by Jurimae and Jurimae <sup>[16]</sup>. Also, this research observed significantly higher skinfold measurements for females than for males when examining children and adolescents ranging from ages 8-18, with an increase in subcutaneous fat with advancing age. The gender difference observed is

consistent with the findings of Westrate *et al.*, <sup>[17]</sup>, Rolland-Cachera *et al.*, <sup>[18]</sup>, Malina *et al.* <sup>[19]</sup> and Dai *et al.*, <sup>[20]</sup>. The probable reason for the accumulations of higher fat in females is in preparation to the enormous energetic demands of reproduction in females and the influence of testosterone, as boys have a significant increase in the growth of bone and muscle with a simultaneous loss of fat in the limbs <sup>[21]</sup>. The maximal loss of fat and increase in muscle mass in the upper arms correspond to the time of peak height velocity as shown especially in the post pubertal males. As height velocity declines, fat accumulation resumes in both genders but is twice as rapid in girls <sup>[21]</sup>. Skinfold thickness allows researchers to distinguish between individuals with high body fat versus high body mass due to lean muscle mass, skeletal composition, which is critical in regards to the potential negative impact on health status <sup>[22]</sup>.

In general, from age 5 to 10 years, boys have 1 to 3 kilograms more fat-free mass (FFM) than girls, but both accrue FFM at similar rates. However, the percentage of body fat is greater in the girls, who have approximately 1% more fat at age 5 years but about 6% by 10 years. During puberty, boys accrue FFM at a much greater rate and for a longer time so that the young adult amount of FFM is attained at age 15 to 16 years for girls, but 19 to 20 years for boys <sup>[23]</sup>. Pubertal girls increase the percentage of body fat and accrue fat mass at a rate of 1.14 kg/year. Pubertal boys decrease the percentage of body fat by 1.15 kg/year, but the fat mass remains relatively constant. As noted above, marked changes in body composition, including alterations in the relative proportions of water, muscle, fat, and bone, are hallmarks of pubertal maturation and the result of

typical female to male differences. Under the influence of the gonadal steroid hormones and the growth hormone, increases in bone mineral content and muscle mass occur, and the deposition of fat is maximally sexually dimorphic <sup>[23]</sup>. The changes in the distribution of body fat (central versus peripheral, subcutaneous versus visceral, upper versus lower parts of the body) result in the typical android and gynoid patterns of fat distribution of the older adolescent and adult <sup>[24]</sup>. Higher amounts of body fat and obesity are associated with increased risks of adverse health events and greater mortality <sup>[25]</sup>. Skinfold thickness are commonly measured in clinical and field settings for the assessment of percentage body fat (%BF) because this method is simple to perform and low in cost <sup>[26]</sup>.

### Conclusion

There is significant sex variation in skinfold thicknesses of Nigerian school children in Kaduna metropolis with age as a major contributor. The females had more subcutaneous fat than the males among all the age categories studied.

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## The Influence of Birth Order on Growth Pattern of Children Aged 5-12 Years from Kazaure Emirate, Jigawa State, Nigeria

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### Abstract

Anthropometry is defined as the measurements of different parts of the body and is widely used in surveys as an indicator of nutritional and health status. Studies revealed that a large number of socio-economic variables are associated with the physical development of children. These variables consist of the parental profession, income, education, birth order, family size, and urbanization. Materials used for the study were Stadiometer, non-elastic measuring tape, Skin fold calliper. The study involved a cross-sectional survey comprising of 863 pupils randomly selected from public primary schools in Kazaure emirate. The subjects were noted for sex and age. A stadiometer was used to measure height to the nearest 0.1 cm and weight to the nearest 0.5 kg. A non-elastic measuring tape was used for the measurement of head, neck, chest, mid-upper arm, hip, waist and calf circumferences to the nearest 0.1cm respectively. A Harpenden skin fold calliper was used for the measurement of biceps and triceps skin fold thicknesses to the nearest 0.1 mm respectively. The study participants were apparently healthy public primary school pupils aged between 5 - 12years, from Kazaure Emirate and of Hausa ethnic group (parents and grandparents are Hausa). Based on birth order between 1-4, males had higher values of height (HT), weight (WT), body mass index (BMI), mid-upper arm circumference (MUAC), head circumference (HDC), neck circumference (NC), chest circumference (CTC), waist (WC) and calf (CC) circumferences than females with a statistical significant difference at  $p < 0.05$ . Females had higher values of biceps (BSF), triceps (TSF) skinfold thicknesses and hip (HC) circumference than males with a statistical difference at  $p < 0.05$ . Based on birth order  $> 4$ , males had higher values of HT, WT, BMI, HDC, NC, CTC, WC and CC than females with a statistical difference at  $p < 0.05$ . Females had higher values of BSF, TSF and HC than males with a statistical difference at  $p < 0.05$ . Males of birth order (1-4) had higher values of HT, WT, BMI, MUAC, HDC, NC, CTC, WC and CC than males of birth order  $> 4$  with a statistical difference at  $p < 0.05$ . However, females of birth order (1-4) had higher values of HT, WT, BMI, MUAC, HDC, NC, and HC than females of birth order  $> 4$  with a statistical difference at  $p < 0.05$ .

**Keywords:** Age, Birth order, Growth, Kazaure emirate, Nigeria

### Introduction

Anthropometry which is defined as the measurements of different parts of the body is widely used in surveys as an indicator of nutritional and health status (1, 2). The normality of human growth and development is internationally recognized as the most sensitive indicator of child health and well-being (3). Nigeria being one of the developing countries, the characteristic pattern of poverty, poor maternal education, high rates of morbidity, and inadequate nutritional intake of the mother and her child combine to produce a pattern of growth characterized by an increased risk of low birth weight, poor growth velocities, and a growth

status that gradually falls away from the norms of children in developed countries (4).

The most important criteria for healthiness and well-being of children are growth status and growth pattern. The analysis of growth patterns and the detection of aberrant growth patterns provide crucial information for the detection of pathologic condition (5). So growth and maturation of children are sensitive indices of health (6, 7) and is influenced by many factors. Socio-economic state (SES) is a concept devised to measure some aspects of education, occupation, and social prestige of a person or a social group (8, 9) observed urban children were taller and grow faster than their rural



peers. Studies revealed that a large number of social-economic variables are associated with the physical development of children. These variables are consisting of the parental profession, income, education (10, 11), birth order (12), family size (13), and urbanization (14).

## Materials and Methods

### Materials

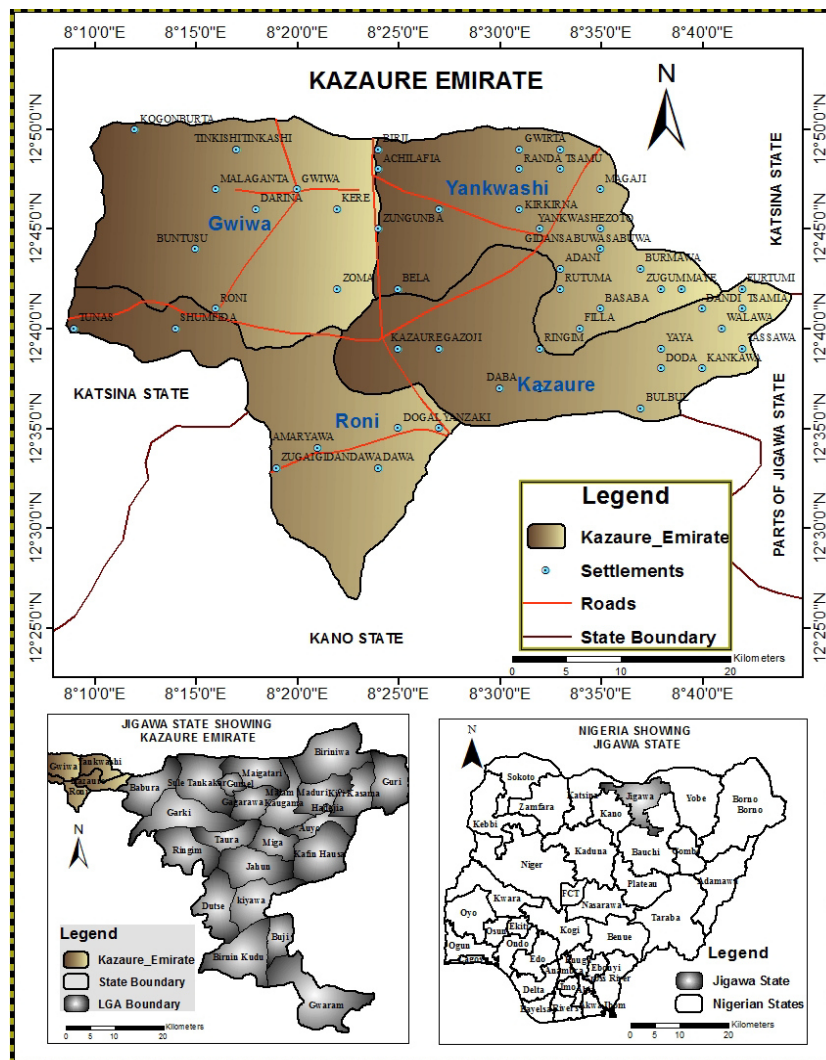
The following materials were used for the study:

- Stadiometer (Holtain Ltd., Crymych, Dyfed, UK)
- Non-elastic measuring tape (Seca 201 Ergonomic Circumference Measuring Tape, Amazon, UK)

- Harpenden skin folds Caliper (Harpenden Skin Fold Caliper, Amazon, UK)

### Design and Study Population

The study design was a cross-sectional survey comprising of 863 subjects. The subjects were noted for sex and age. The study was carried out in four (4) Local Government Areas (LGAs) of Jigawa State. Namely: Kazaure, Roni, Gwiwa and Yankwashi Local Governments Areas respectively. Of the four (4) LGA's, three towns and related primary schools were randomly selected and the participants were measured and the values recorded.



**Figure 1:** Map of Kazaure emirate. Modified from the administrative map of Nigeria



### Study Area

Kazaure is located in the Northern part of Jigawa State, one of the 27 local governments in Jigawa State. It lies between longitude 12° 30' to 12° 45' and latitude 8° 15' to 8° 30' North and East respectively. It covers a land area of about 1780 kilometres square. It is bordered to the north by Daura (Katsina State), West by Ingawa (Katsina State), and to the South by Dambatta (Kano state) (15, 16). The area belongs to the Sudan Savanna Vegetation. Rainfall begins between May and June and ends around September and October. The main annual rainfall is about 600mm with the highest input during the months of July and August. The mean annual temperature is about 26 °C but means monthly value ranges between 22° C in the coldest months (December and January) and 31°C in the hottest months of April and May (15).

### Inclusion criteria

Participant must be:

- i. public primary school pupil
- ii. between 5-12 years of age
- iii. apparently physically/mentally fit
- iv. from Kazaure Emirate
- v. Hausa ethnic group (parents and grandparents are Hausa)

### Exclusion criteria

Participant must not be:

- i. private primary school pupil
- ii. below 5 years or above 12 years, of age
- iii. physically/ mentally unfit or deformed
- iv. from any Emirate other than Kazaure Emirate
- v. of any ethnic group other than Hausa

### Ethical Approval (Appendix I)

#### Methods of Anthropometric Assessment

A stadiometer was used to measure height to the nearest 0.1 cm and weight to the nearest 0.5 kg, a non-elastic measuring tape was used for the measurement of head, neck, chest, mid-upper arm, hip, waist and calf circumferences respectively to the nearest 0.1cm, a Harpenden skin fold calliper was used for the measurement of biceps and triceps skin fold thicknesses respectively to the nearest 0.1 mm (17, 18).

- i. **Height/ Stature (HT):** The subjects stood up perfectly upright with arms relaxed by the side, and ankles and knees placed together. The subjects were encouraged to stand as upright as possible and bare footed before measurements were taken. The stadiometer was positioned behind the subjects and the measurement taken to the nearest 0.1cm.
- ii. **Body weight:** This was taken with subjects wearing light clothes and barefooted to the nearest 0.5kg.
- iii. **Mid upper arm circumference (MUAC):** This was measured using the tailor's plastic measuring tape at the midpoint of the upper non-dominant arm, between the acromial process and the tip of the olecranon and recorded to the nearest 0.1cm.
- iv. **Head/occipitofrontal circumference (HDC):** This was measured using plastic tape from the occiput of the skull to the most anterior portion of the frontal bone and recorded to the nearest 0.1cm.
- v. **Hip circumference (HC):** This was measured with the subject in standing position. The tailor's plastic tape was placed on the tip of the buttock's curvature posteriorly and pubis anteriorly. The measurement was to the nearest 0.1cm.
- vi. **Waist circumference (WC):** This was taken while subject in standing position. The tape was placed between the lowest lumbar region and the sacral region posteriorly and pubic region anteriorly. The measurement was to the nearest 0.1cm.
- vii. **Calf circumference (CC):** This was taken with the subject in standing position. The tape was placed on the gastrocnemius muscle posteriorly and the tibial bone anteriorly. The measurement was to the nearest 0.1cm.
- viii. **Neck circumference (NC):** The tape was placed around the neck measurement recorded to the nearest 0.1 cm.
- ix. **Chest circumference (CTC):** The tape was placed around the chest with the tape meeting on the sternum and measurement recorded to the nearest 0.1 cm.

- x. **Biceps skin fold thickness (BSF):** This was measured on the left side using the Harpenden calliper. The measurement was to the nearest 0.1 mm at the upper non-dominant arm anteriorly.
- xi. **Triceps skin folds thickness (TSF):** This was measured using the Harpenden skin fold caliper at the midpoint of the upper non-dominant arm posteriorly to the nearest 0.1mm. All skin fold measurements were taken twice and the average recorded.

### Statistical Analyses

Data were expressed as mean  $\pm$  standard deviation (SD). Differences between boys and girls were tested using the Student's t-test. One-way analysis of variance (followed by Benferoni Post Hoc test) was used to investigate the effect of socio-economic factor (birth order) on the different anthropometric parameters. The statistical significant difference was deemed acceptable at  $P < 0.05$ . The data were analyzed using Statistical Package for Service Solutions (SPSS) version 20 (IBM Corporation, Armonk, New York, USA).

### Results

Table 1 shows the descriptive statistics of male and female pupils who participated in study. The results showed the Mean  $\pm$  SD, minimum and maximum values of height, weight, body mass index, biceps and triceps skinfold thicknesses; mid-upper arm, head, neck, chest, waist, hip and calf circumferences of the pupils.

Table 2 Based on birth order (1-4) male had higher values of all the parameters than females, with the exception of BSF, TSF and HC with statistical significant difference at  $p < 0.05$ . Moreover, based on birth order ( $>4$ ) males had higher values of all the parameters than females with the exception of BSF, TSF and HC, with statistical difference at  $p < 0.05$ .

Table 3 Males of birth order (1-4) had higher values of all the parameters than those of birth order  $>4$ . However, all the variables were statistically significant at  $p < 0.05$  with the exception of BSF, TSF, CC and HC. Furthermore, females of birth order (1-4) had higher values of all the parameters than those of birth order ( $>4$ ). However, all the variables were statistically significant at  $p < 0.05$ , with the exception of BSF, TSF, CTC, WC and CC.

**Table 1:** Descriptive Statistics of Male and Female Pupils From Kazaure Emirate (n=863)

| Variables                            | Male (n= 432)      |               | Female (n= 431)    |              |
|--------------------------------------|--------------------|---------------|--------------------|--------------|
|                                      | Mean $\pm$ SD      | Min – Max     | Mean $\pm$ SD      | Min-Max      |
| Age (years)                          | 8.56 $\pm$ 2.27    | 5.00-12.00    | 8.43 $\pm$ 2.34    | 5.00-12.00   |
| Height (cm)                          | 124.00 $\pm$ 14.00 | 99.00 -150.00 | 121.00 $\pm$ 14.00 | 96.00-148.00 |
| Weight (kg)                          | 25.14 $\pm$ 4.07   | 10.9 0-40.30  | 23.60 $\pm$ 4.83   | 13.30-37.30  |
| Body Mass Index (kg/m <sup>2</sup> ) | 15.64 $\pm$ 1.52   | 12.27-21.44   | 14.24 $\pm$ 1.41   | 11.54-20.08  |
| Biceps Skinfold Thickness (mm)       | 1.17 $\pm$ 0.26    | 1.00-2.00     | 1.46 $\pm$ 0.21    | 1.00-2.54    |
| Triceps Skinfold Thickness (mm)      | 1.12 $\pm$ 0.33    | 1.00-2.00     | 1.41 $\pm$ 0.22    | 1.00-2.50    |
| Mid-upper arm Circumference (cm)     | 16.80 $\pm$ 1.39   | 12.50-22.00   | 15.72 $\pm$ 1.62   | 11.50-20.00  |
| Head Circumference (cm)              | 50.73 $\pm$ 2.41   | 48.00-56.30   | 49.26 $\pm$ 2.67   | 47.00-54.00  |
| Neck Circumference (cm)              | 24.89 $\pm$ 1.34   | 20.50-32.50   | 23.33 $\pm$ 1.66   | 19.00-30.00  |
| Chest Circumference (cm)             | 57.62 $\pm$ 4.37   | 44.50-68.50   | 55.73 $\pm$ 4.20   | 42.60-67.00  |
| Waist Circumference (cm)             | 56.14 $\pm$ 3.94   | 46.00-69.50   | 54.82 $\pm$ 4.57   | 42.50-66.00  |
| Hip Circumference (cm)               | 59.50 $\pm$ 5.53   | 44.20-72.00   | 62.80 $\pm$ 4.94   | 48.20-75.00  |
| Calf Circumference (cm)              | 22.55 $\pm$ 1.98   | 12.80-28.00   | 21.83 $\pm$ 2.51   | 12.00-27.00  |

**Table 2:** Influence of Birth Order on Height, Weight, BMI and Body Circumferences of Pupils Aged 5-12 Years from Kazaure Emirate

| Variables                | Birth order 1-4               |                                 |       |         | Birth order >4                |                                |       |         |
|--------------------------|-------------------------------|---------------------------------|-------|---------|-------------------------------|--------------------------------|-------|---------|
|                          | Male (n=295)<br>Mean $\pm$ SD | Female (n=302)<br>Mean $\pm$ SD | F     | p-value | Male (n=137)<br>Mean $\pm$ SD | Female(n=129)<br>Mean $\pm$ SD | F     | p-value |
| HT (cm)                  | 124.68 $\pm$ 13.44            | 122.17 $\pm$ 13.51              | 2.789 | 0.001   | 123.14 $\pm$ 13.67            | 121.46 $\pm$ 13.75             | 2.734 | 0.001   |
| WT (kg)                  | 25.94 $\pm$ 1.65              | 24.51 $\pm$ 1.70                | 2.953 | 0.001   | 24.54 $\pm$ 1.61              | 23.85 $\pm$ 1.68               | 2.297 | 0.01    |
| BMI (kg/m <sup>2</sup> ) | 15.59 $\pm$ 1.57              | 14.61 $\pm$ 1.43                | 3.350 | 0.001   | 15.25 $\pm$ 1.39              | 14.57 $\pm$ 1.41               | 2.392 | 0.01    |
| BSF (mm)                 | 1.17 $\pm$ 0.26               | 1.73 $\pm$ 0.25                 | 1.976 | <0.05   | 1.16 $\pm$ 0.30               | 1.68 $\pm$ 0.24                | 2.509 | 0.01    |
| TSF (mm)                 | 1.41 $\pm$ 0.34               | 1.87 $\pm$ 0.19                 | 1.980 | <0.05   | 1.39 $\pm$ 0.43               | 1.75 $\pm$ 0.31                | 1.879 | <0.05   |
| MUAC (cm)                | 17.91 $\pm$ 1.37              | 16.86 $\pm$ 1.65                | 2.261 | 0.01    | 16.79 $\pm$ 1.41              | 16.73 $\pm$ 1.55               | 0.994 | 0.321   |
| HDC (cm)                 | 51.02 $\pm$ 2.74              | 50.51 $\pm$ 2.10                | 3.061 | 0.001   | 50.42 $\pm$ 2.40              | 49.45 $\pm$ 2.51               | 2.073 | 0.01    |
| NC (cm)                  | 24.87 $\pm$ 1.35              | 23.71 $\pm$ 1.72                | 1.886 | <0.05   | 23.91 $\pm$ 1.31              | 23.48 $\pm$ 1.54               | 1.990 | <0.05   |
| CTC (cm)                 | 57.78 $\pm$ 2.26              | 55.74 $\pm$ 2.25                | 5.159 | <0.001  | 56.57 $\pm$ 2.12              | 55.65 $\pm$ 2.15               | 2.477 | 0.01    |
| WC (cm)                  | 55.79 $\pm$ 2.82              | 54.75 $\pm$ 2.58                | 2.166 | 0.01    | 54.73 $\pm$ 2.29              | 54.01 $\pm$ 2.45               | 1.883 | <0.05   |
| HC (cm)                  | 59.24 $\pm$ 2.51              | 60.28 $\pm$ 2.69                | 2.128 | 0.01    | 59.15 $\pm$ 2.27              | 60.19 $\pm$ 2.20               | 2.134 | 0.01    |
| CC (cm)                  | 22.68 $\pm$ 1.99              | 21.79 $\pm$ 1.54                | 2.030 | 0.01    | 22.59 $\pm$ 1.36              | 21.65 $\pm$ 1.47               | 2.125 | 0.01    |

HT=height, WT= Weight, BMI= Body mass index, BSF=Biceps skinfold thickness, TSF= Triceps skinfold thickness, MUAC= Mid-upper arm circumference, HDC= Head circumference, NC= Neck circumference, CTC= Chest circumference, WC= Waist circumference, HC= Hip circumference, CC= Calf circumference

**Table 3:** Influence Of Birth Order 1-4 and >4 Within Sex on Height, Weight, Bmi and Body Circumferences of Pupils Aged 5-12 Years from Kazaure Emirate

| Variables                    | 1-4                      | >4                       | F     | P-value | 1-4                        | >4                        | F     | p-value |
|------------------------------|--------------------------|--------------------------|-------|---------|----------------------------|---------------------------|-------|---------|
|                              | Male (n=295)<br>Mean ±SD | Male (n=302)<br>Mean ±SD |       |         | Female (n=137)<br>Mean ±SD | Female(n=129)<br>Mean ±SD |       |         |
| <b>HT (cm)</b>               | 124.68±13.44             | 123.14±13.67             | 2.767 | 0.001   | 122.17±13.51               | 121.46±13.75              | 2.234 | 0.01    |
| <b>WT (kg)</b>               | 25.94±1.65               | 24.54±1.61               | 2.744 | 0.001   | 24.51±1.70                 | 23.85±1.68                | 2.217 | 0.01    |
| <b>BMI(kg/m<sup>2</sup>)</b> | 15.59±1.57               | 14.55±1.39               | 2.752 | 0.001   | 15.31±1.43                 | 14.47±1.41                | 2.252 | 0.01    |
| <b>BSF (mm)</b>              | 1.17±0.26                | 1.16±0.30                | 0.676 | 0.345   | 1.73±0.25                  | 1.68±0.24                 | 1.409 | 0.311   |
| <b>TSF (mm)</b>              | 1.41±0.34                | 1.39±0.43                | 0.580 | 0.501   | 1.87±0.19                  | 1.75±0.31                 | 1.537 | 0.305   |
| <b>MUAC (cm)</b>             | 17.91±1.37               | 16.79±1.41               | 2.311 | 0.01    | 16.86±1.65                 | 16.23±1.55                | 1.894 | <0.05   |
| <b>HDC (cm)</b>              | 51.02±2.74               | 50.42±2.40               | 2.001 | 0.01    | 50.51±2.10                 | 49.45±2.51                | 2.056 | 0.01    |
| <b>NC (cm)</b>               | 24.87±1.35               | 23.91±1.31               | 1.984 | <0.05   | 23.71±1.72                 | 23.18±1.54                | 1.890 | <0.05   |
| <b>CTC (cm)</b>              | 57.78±2.26               | 56.57±2.12               | 3.012 | <0.001  | 55.74±2.25                 | 55.65±2.15                | 1.377 | 0.521   |
| <b>WC (cm)</b>               | 55.79±2.82               | 54.73±2.29               | 2.175 | 0.01    | 54.75±2.58                 | 54.01±2.45                | 1.483 | 0.515   |
| <b>HC (cm)</b>               | 59.24±2.51               | 59.15±2.27               | 0.765 | 0.401   | 60.28±2.69                 | 59.39±2.20                | 2.204 | 0.01    |
| <b>CC (cm)</b>               | 22.68±1.99               | 22.59±1.36               | 0.530 | 0.435   | 21.79±1.54                 | 21.65±1.47                | 1.031 | 0.674   |

HT=height, WT= Weight, BMI= Body mass index, BSF=Biceps skinfold thickness, TSF= Triceps skinfold thickness, MUAC= Mid-upper arm circumference, HDC= Head circumference, NC= Neck circumference, CTC= Chest circumference, WC= Waist circumference, HC= Hip circumference, CC= Calf circumference

### Discussion

The anthropometric parameters of growth examined in this study population showed that the males had higher height, weight and BMI than females. Studies by (2, 19, 20) showed that males had higher height, weight and BMI than the females. Additionally, studies by (21, 22, 23) reported that males were taller than the females respectively. However, studies by (24) showed that the females were found to be taller, heavier and had higher BMI than the males. This differs from the result of this study in which the males were taller, heavier and had higher BMI. Similarly, females had higher values of biceps and triceps skin folds thicknesses than the males, and this agrees with the findings of (2) which stated that females had higher values for triceps and subscapular skin folds than

the males at all ages (12 – 17 years). The males had higher values of body circumferences than the females, except for the hip circumference. This is supported by (2) who stated that the males had higher waist circumference than the females while the females had higher mid-upper arm and hip circumferences than the males. Studies by (23) reported that Igbo males had higher head circumference than the Igbo females. Likewise, males had higher waist and neck circumferences than the females and this is in accordance with the findings of (25).

Studies by (26,27, 28, 29) concluded that children belonging to higher and middle socioeconomic groups are larger in body size than those in lower socio-economic groups. Studies by (30, 31, 32) showed that an increase in anthropometric measurements is associated to families with the higher education and income levels. This agrees with

the findings of this study that showed children of low birth order with better growth pattern than those of higher birth order. Eiben *et al.* (12) evaluated the effect of socio-economic status on weight and height of children. Their results showed that people of high economic status had more height than low economic status. They also observed that children of higher salary earners were taller than unskilled worker about 2.9 cm (12). Moreover, this study is in accordance with other studies that opined that children of lower economic status have lower weight and height (11).

### Conclusion

This study observed that children of low birth order showed better growth than those of higher birth order which reflects larger family size. Therefore, the smaller the family size, the better the growth of the children.

**Conflict of Interest:** None declared

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## Vitamin D Supplementation Ameliorated Fructose-induced Dyslipidaemia in Male Wistar Rats

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### Abstract

The effect of diabetes mellitus on lipid metabolism is well established. Animal studies suggest that vitamin D supplementation preserved pancreatic  $\beta$ - cells, improves insulin synthesis and sensitivity and restores normal lipid profiles. The study was designed to evaluate the effects of vitamin D administration on lipid profiles and cardiovascular risk indices in fructose-fed male rats. The rats were randomised in to four groups of five animals each. Group I: Control (C); received distilled water, Group II: Vitamin D (VD); received vitamin D, Group III: Fructose-drinking rats (FDR-10); received fructose, Group IV: FDR-10 + VD; received fructose + vitamin D. Fructose-fed rats exhibited significant increase in serum total cholesterol ( $4.60 \pm 0.23$  vs  $3.88 \pm 0.19$  mmol/L), triglycerides ( $1.90 \pm 0.27$  vs  $1.20 \pm 0.16$  mmol/L), and LDL-cholesterol ( $2.80 \pm 0.26$  vs  $2.16 \pm 0.17$  mmol/L) while high density lipoprotein cholesterol was not significantly decreased compared to the control ( $1.22 \pm 0.07$  vs  $1.32 \pm 0.22$  mmol/L). In addition, fructose-fed rats showed marked increase in total cholesterol/high density lipoprotein cholesterol and low density lipoprotein cholesterol/high density lipoprotein cholesterol ratios. Compared to fructose-fed rats, vitamin D treated fructose-fed rats exhibited significant decreased in serum TC ( $3.86 \pm 0.11$  vs  $4.60 \pm 0.23$  mmol/L), TG ( $1.30 \pm 0.16$  vs  $1.90 \pm 0.27$  mmol/L), and LDL-C ( $2.20 \pm 0.40$  vs  $2.80 \pm 0.26$  mmol/L) while HDL-C was not significantly increased ( $1.40 \pm 0.16$  vs  $1.22 \pm 0.07$  mmol/L). Oral vitamin D treatment restored fructose-induced dyslipidaemia and cardiovascular risk indices in fructose-fed rats.

**Keywords:** *Dyslipidaemia, Type 2 diabetes mellitus, Vitamin D, Pancreatic  $\beta$ -cells, insulin sensitivity, Cardiovascular risk indices*

### Introduction

The incidence of diabetes is increasing globally and type 2 diabetes mellitus (T2DM) and its complications constitute a major public health problem [1]. In Nigeria, diabetes mellitus affects about 2.8 millions adults between the ages of 20-79 with gender distribution of 1.5 males and 1.3 females [2]. Studies have shown that almost 90% of T2DM cases are attributed to five major factors; poor dietary choices, physical inactivity, obesity, smoking and alcohol consumption [3, 4]. Nutritional factor is particularly important for the fact that T2DM is associated with dysfunctional metabolism and utilisation of carbohydrates, fats and proteins. Beta-cells dysfunction or loss in pancreatic islets leads to hyperglycaemia and hyperlipidaemia [5].  $\beta$ -cell dysfunction, decreased insulin sensitivity and

systemic inflammation are common indicators of T2DM [6]. High fructose-feeding is associated with the induction of metabolic dyslipidaemia providing an animal model of insulin resistance, metabolic syndrome or T2DM [7, 8, 9]. The dyslipidaemia may be attributed to increased de novo lipogenesis and fatty acid synthesis [10]. In animals studies, vitamin D supplementation enhances insulin secretion and insulin sensitivity [11, 12] and also promotes  $\beta$ -cell survival by attenuating the generation and effects of inflammatory cytokines [13, 14]. Amelioration of insulin resistance, islet dysfunction and systemic inflammation are important in the treatment of T2DM. Vitamin D may be a potential nutritional factor in attenuating metabolic dyslipidaemia and its associated complications in male albino rats. The aim of the study was to investigate the effects of

vitamin D supplementation on lipid profiles in fructose-fed rats.

## Materials and Methods

### Experimental animals

A total of thirty five (35) adult male Wistar rats, weighing between 150 – 250 g were obtained from the Department of Human Physiology, Faculty of Basic Medical Sciences, Gombe State University. The animals were housed in well-aerated laboratory cages in the animal's house of the Department of Human Physiology. The experimental animals were provided with growers mash from Vital Feeds Company Kaduna Nigeria and were allowed free access to water *ad-libitum*.

### Drugs and Chemicals

All chemicals and drugs used in the study were of analytical grade and were obtained from PSPARK, Scientific limited Northampton, UK.

### Preparation and administration of vitamin D

Vitamin D was dissolved in carboxymethylcellulose and was orally administered for eight weeks.

### Induction of dyslipidaemia in experimental albino rats

The experimental rats were fed 10% fructose solution in drinking water for five (5) weeks as described [14].

### Experimental design and treatment schedule

Animals were randomised into four groups of five animals each and vitamin D was orally administered at a dose of 5000IU/kg. The animals were subjected daily to the following treatment schedule.

Group I: Control (C); received distilled water

Group II: Vitamin D (VD); received vitamin D

Group III: Fructose-drinking rats (FDR-10); received fructose

Group IV: FDR-10 + VD; received fructose + vitamin D (vitamin D treatment began five weeks after the commencement of fructose feeding)

### Collection and processing of blood samples

Eight weeks after the commencement of the treatment, overnight fasted rats were sacrificed and blood samples were collected by cardiac puncture in centrifuge tubes as described by Mallick [15]. The

blood samples were centrifuged at 3000 rpm for 15 min (Denley BS400; England) to obtain clear serum for biochemical analysis.

### Determination of lipid Profiles and cardiovascular risk factors

Lipid profiles; total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) levels were determined using commercial Span diagnostic reagent kits (BRIC, BARC, Mumbai, India) according to the instructions of the manufacturer. The results were expressed in mmol/L. Cardiovascular risk indices were calculated according to Ross [16] as follows: Total cholesterol/High density Lipoprotein-cholesterol; Low density lipoprotein cholesterol/High density lipoproteins cholesterol.

### Statistical Analysis

Data were expressed as mean  $\pm$  SEM. Data were analysed using SPSS, version 20.0 and all comparisons were made by one way ANOVA test followed by Turkey-Kramer post-hoc test analysis and  $p < 0.05$  was considered significant.

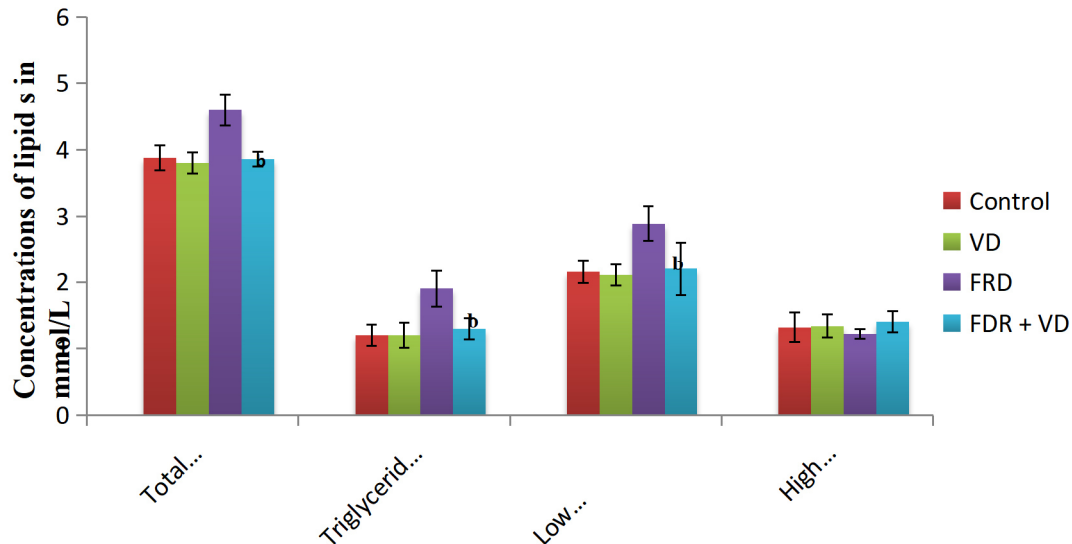
### Results

The effects of fructose-drinking and vitamin D administration on lipid profiles and cardiovascular risk indices are presented graphically in Figures 1 and 2). Fructose-fed rats exhibited significant ( $p < 0.01$ ) increase in serum total cholesterol ( $4.60 \pm 0.23$  vs  $3.88 \pm 0.19$  mmol/L) compared to control. Similarly, fructose feeding resulted in significant elevation ( $p < 0.001$ ) in plasma triglycerides levels ( $1.90 \pm 0.27$  vs  $1.20 \pm 0.16$  mmol/L) compared to the control. In addition, fructose feeding led to a significantly higher ( $p < 0.05$ ) LDL-C level when compared with the control ( $2.80 \pm 0.26$  vs  $2.16 \pm 0.17$  mmol/L). Fructose-feeding caused a reduction in HDL-C level which was not significant ( $p > 0.05$ ) compared to the control ( $1.22 \pm 0.07$  vs  $1.32 \pm 0.22$  mmol/L). Fructose feeding produced dyslipidaemia in fructose-drinking rats. In addition, fructose-drinking rats showed marked increase in TC/HDL-C ( $p < 0.001$ ) and LDL-C/HDL-C ratios ( $p < 0.001$ ) indicating the negative effects of fructose on cardiovascular system. Compared to the fructose-fed rats, rats supplemented with vitamin D exhibited significant ( $p < 0.01$ ) decreased in serum total

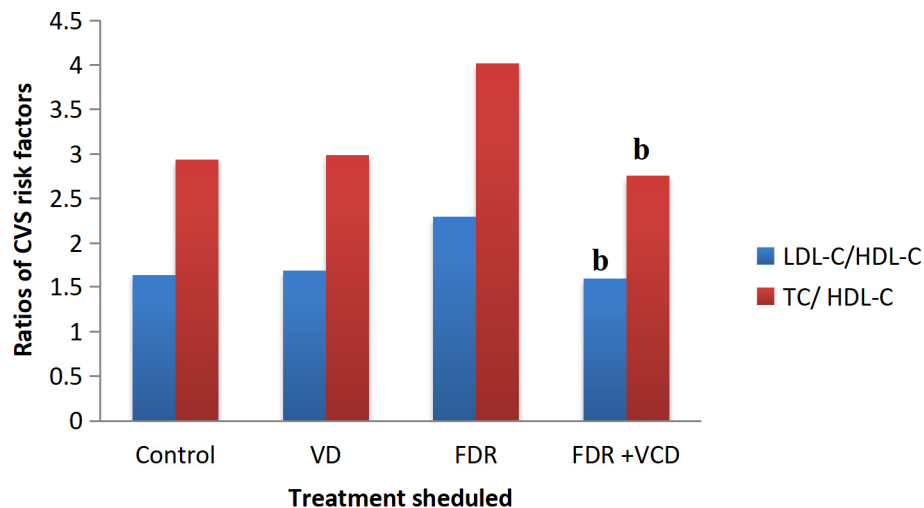


cholesterol ( $4.60 \pm 0.23$  vs  $3.86 \pm 0.11$  mmol/L). Also, in fructose-fed rats, treatment with vitamin D led to a significantly lowered ( $p < 0.001$ ) serum triglyceride level when compared to the control ( $1.30 \pm 0.16$  vs  $1.90 \pm 0.27$  mmol/L). In addition, LDL-

C was significantly reduced ( $p < 0.05$ ) in fructose-fed vitamin D supplemented rats ( $2.20 \pm 0.40$  vs  $2.80 \pm 0.26$  mmol/L). In fructose-fed rats, supplementing with vitamin D led to a higher but none significant HDL-C level ( $1.40 \pm 0.16$  vs  $1.22 \pm 0.07$  mmol/L).



**Figure 1:** Effects of vitamin D treatment on lipid profiles in fructose drinking rats. Results are expressed as mean  $\pm$  SEM (N = 5). **b** indicates levels of significant difference ( $P \leq 0.05$ ) VD compared to fructose fed rats. Control, VD = vitamin D, FRD = Fructose drinking rats.



**Figure 2:** Shows the effects of vitamin D supplementation on cardiovascular risk factors in normal and fructose-fed rats.

(Values are presented as mean  $\pm$  SEM (N = 5). **b** = significant difference in LDL-C/HDL-C and TC/HDL-C ratios in VD treated compared to FDR group. VD = vitamin D, FRD = Fructose drinking rats)

## Discussion

The results of the study revealed that vitamin D supplementation in fructose-fed rats resulted in significant reduction of total cholesterol, triglyceride, and low density lipoprotein cholesterol with no significant increase in high density lipoproteins cholesterol. Thus, vitamin D treatment corrected the observed alteration in lipid profiles and atherogenic indices suggesting the hypocholesterolemic and hypolipidemic effect of vitamin D in fructose fed rats. Vitamin D counteraction of fructose-induced increase in these parameters strongly supports the notion that vitamin D supplementation might be useful in attenuating some of the diabetic complications as well as reduce the risk of developing cardiovascular diseases [17]. The effects of previous vitamin D supplementation studies on glycemic control, insulin resistance and dyslipidaemia are highly controversial. Consistent with our findings, studies have shown that vitamin D supplementation is associated with improvement in lipid profiles [18, 19, 20] and protection against T2DM [21]. However, other studies demonstrated no improvement in lipid profiles after vitamin D supplementation.[22, 23] The mechanism by which vitamin D consumption ameliorated fructose-induced dyslipidaemia in rats is not fully elucidated but could be attributed to increased synthesis and enhancement of the sensitivity of target tissues to circulating insulin through the regulation of insulin receptor gene and also its effects on calcium and phosphorus metabolism [20]. In addition, vitamin D treatment has been shown to inhibit lipid synthesis and prevent fatty acid induced insulin resistance [14]. It has been demonstrated that improved vitamin D level has been shown to reduce the risk factor for atherosclerosis and protects against increased cardiovascular risk factors [24, 25, 26]. Moreover, studies have shown that vitamin D supplementation attenuates the production of pro-inflammatory cytokines in diabetic patients due to its antioxidant, immunomodulatory and anti-inflammatory effects [27,

13]. Protection of pancreatic  $\beta$ -cells by suppressing oxidative stress and down regulating inflammatory cytokines production could improve serum insulin levels which enhances adiponectin levels which inhibits the expression of pro-inflammatory cytokines TNF- $\alpha$  and IL-6 in various tissues [28].

In summary, the result of the present study indicates the beneficial effects of the vitamin D supplementation in improving the imbalance in lipoprotein metabolism in fructose-induced dyslipidaemia in male albino rats.

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