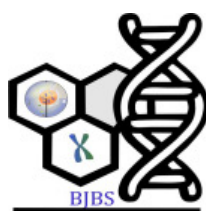


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Bayero Journal of Biomedical Sciences (BJBS) is a journal that publishes original research and critical reviews in the fields of Anatomy, Biochemistry, Microbiology, Nutrition and Dietetics, Pharmacology and Physiology. It is a biannual publication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

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Acknowledgements

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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 4, 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 biannual publication of the Faculty of Basic Medical Sciences, Bayero University, Kano, Nigeria. It 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 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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Bleeding and Clotting Time During Menstrual Phase among Hausa-Fulani Secondary School Students at Fagge Local Government Area, Kano State, Nigeria

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Abstract

Menstruation is an orderly cyclic shedding of the endometrial lining that occurs in response to the interactions of hormones produced by the hypothalamus, pituitary gland, and the ovaries. Various phases of menstruation are associated with changes in thrombotic and fibrinolytic activities that can be assessed using bleeding and clotting times. **Aim:** The aim of this study was to determine the mean values of bleeding and clotting times among young adolescent females during their menstrual phase. **Method and Materials:** the study was conducted at Mukaddami Secondary School, Fagge Local Government area of Kano state between August 12th to September 13th, 2018. One hundred and twenty adolescent girls in their menstrual phase were recruited as the study group while sixty nine girls who were in other phases of the menstrual cycle were recruited as controls. A structured questionnaire was used to obtain sociodemographic information of the subjects. Bleeding time was assessed using Duke's method while clotting time was assessed using the capillary tube method. Data were analysed on Statistical Package for Social Scientists (SPSS) version 20.0 using students' t-test and Pearson's correlation; $p\text{-value} \leq 0.05$ was considered statistically significant. **Results:** The mean age in years of the study group was 16.48 ± 1.13 while that of control group was 16.74 ± 1.22 . There was no statistically significant difference in the age of the subjects in both groups ($p = 0.15$). The average age at menarche for both study and control groups was 12.38 ± 0.94 , and 12.93 ± 1.46 , respectively and difference was statistically significant ($p = 0.01$). The study group had statistically significant shorter bleeding time compared to the control group ($129.63 \text{ sec} \pm 47.74$ and $234.78 \text{ sec} \pm 38.49$, respectively, $p = 0.01$). Similarly, the study group had statistically significant shorter clotting time compared to the controls ($175.67 \text{ sec} \pm 42.12$ and $404.06 \text{ sec} \pm 60$, respectively, $p = 0.01$). About 50% of the subjects in the study group use 2 pads per day while 42% used at least 3 pads per day. Those that used 1 pad per day had significantly ($p = 0.01$) longer clotting time ($240.00 \text{ sec} \pm 00$) compared to those that used 2 pads ($205.15 \text{ sec} \pm 24.1$) or at least 3 pads ($135.10 \text{ sec} \pm 22.4$) per day. There was no statistically significant difference in bleeding time among the study subjects based on the number of pads used per day ($p = 0.24$). As the number of pads used per day increases both bleeding time ($r = -0.54$, $p < 0.05$) and clotting time ($r = -0.82$, $p < 0.05$) decrease. **Conclusion:** Adolescent girls in their menstrual phase have statistically significant shorter bleeding and clotting times compared to those in other phases of menstrual cycle. Further studies should look at the serum levels of bleeding and clotting markers in adolescent girls in their menstrual phase.

Keywords: Adolescent, Girls, Menstrual Phase, Bleeding, Clotting, Time

Introduction

Menstruation is an orderly cyclic shedding of the endometrial lining that occurs in response to the interactions of hormones produced by the hypothalamus, pituitary gland, and the ovaries. The menstrual cycle is essential for the production of eggs, and for the preparation of the uterus for pregnancy.^[1] In the absence of pregnancy, steroid hormone levels begin to fall due to declining corpus luteum function.^[2] This hormonal withdrawal results

in increased coiling and constriction of the spiral arterioles, which eventually results in tissue ischemia as a result of reduced blood flow to the superficial layer of the endometrium. The endometrium releases prostaglandins that cause contractions of the uterine smooth muscle and sloughing of the degraded endometrial tissue.^[2] Thus, every cycle has two opposing hemostatic vascular responses of pro-coagulants and anti-coagulants

processes in order to maintain endometrial hemostasis. The pro-coagulants are the platelets, endothelial factors and soluble clotting factors inside the blood, while the anti-coagulants include the antithrombin III, protein C, protein S and the fibrinolytic system. The menstrual fluid contains desquamated endometrial tissue, red blood cells, prostaglandins, and fibrinolysin. The fibrinolysin lyses clots, so that menstrual blood does not normally contain clots unless the flow is excessive.^[3] The menstrual process involves two cycles, namely; ovarian and uterine cycles based on changes taking place in both the ovaries and the uterus respectively.^[4] The ovarian cycle comprises three phases; follicular, ovulation, and luteal phases, while the uterine cycle, is made up of the menstrual, proliferative, ovulatory and secretory phases. Neuro-endocrine reflexes control both cycles in order to maintain the normal hemostasis. Studies conducted during menstruation show fibrinogen level, coagulation factors; II, VII, X and platelet retention to be lower in the menstrual phase than in luteal phase and fibrinolytic activity to be higher in menstrual phase.^[5] It is obvious that the interplay between clotting and fibrinolytic systems will determine the bleeding and clotting times during each menstrual cycle. The aim of this study was to determine the mean values of bleeding and clotting times among young adolescent females during their menstrual phase and compare with those outside the menstrual phase who served as controls in order to determine quantitative differences if any in these values so as to provide a local reference data for use in this part of the country.

Materials and Methods

Setting

The study was conducted at Mukaddami Secondary School located in Fagge Local Government Area of Kano State. The school has a total population of 645 students comprising of two separate sections; Arabiyya and the normal secular sections. Each section is made up of six different arms of classes that span from junior secondary section (JS) to senior secondary section (SS). The junior classes comprise of JS1 – JS3, while the senior section comprises of SS1 – SS3.

Study Group

This involves 189 young adolescent females divided

into two different groups. The study group who were within their menstrual phase, comprised of 120 students and the control group who were not in their menstrual periods and were at different phases of the menstrual cycle, were 69 students. The study was carried out in the Science laboratory of the school, over a period of one month; from August 12th to September 13th 2018.

Study Design, Sample Size, and Sampling

This is a descriptive cross-sectional study. A multi-stage sampling method was applied to select subjects proportionately by assigning a numeric value of 10 students from each class under each section (Arabic and secular). Thereafter, a random sampling method was used to arrive at the required sample size of 120 students. Balloting method was used as a method of recruiting the cohorts. All those that picked yes were included in the study. The 69 students who served as controls were randomly selected across the classes.

Inclusion criteria

- ❖ Females within the age range of 15 to 20 yrs
- ❖ Those who grant their consent and or assent.

Exclusion Criteria

- ❖ Recent history of viral hemorrhagic fever
- ❖ History of chronic liver disease
- ❖ History of epistaxis and haematological diseases
- ❖ Those on Non-steroidal anti-inflammatory drugs
- ❖ Those with a history suggestive of pelvic inflammatory diseases
- ❖ History of three consecutive irregular menstrual flow
- ❖ Those on hormonal contraceptives
- ❖ Other ethnicities that are not Hausa-Fulani

Ethical Consideration

Ethical approval was obtained from the Ethical and Scientific Committee of the Kano State Ministry of Health (MOH/Off/797/T.I/1078). Approval was obtained from the principal of the school for the students to participate in the study. Thereafter, an informed written consent/assent for those less than 18 years was obtained from the students prior to the commencement of the study.

Data Collection

The procedure was well explained to all the participants. A structured questionnaire was used to document the information obtained from respondents. The questionnaire had three (3) sections that documented information on the respondents' bio-data, menstrual history and actual values of bleeding and clotting times of all the respondents.

Laboratory Procedure

Bleeding Time (BT):

BT was estimated by Duke's method^[6] using Whatman filter paper. The principle involves applying a prick to the skin with a needle, thereafter bleeding occurs for sometimes and then stops. The time interval between the skin puncture and the cessation of bleeding is termed "bleeding time".

The fingertip was sterilised using methylated spirit and air-dried. A deep prick was made using a sterile lancet so that the blood came out freely without squeezing the site of the puncture. The time period required to stop the bleeding from the punctured site was noted by the use of a stopwatch. The blood was wiped off by touching the fingertip with filter paper after every 30 seconds, till bleeding stops. Bleeding time of each subject was calculated using a formula: Number of spots x 30 seconds. Bleeding time was recorded in seconds.

Clotting time (CT): CT was estimated using a capillary tube method. The Principle involves collecting blood in a non-heparinized capillary tube until it coagulates, forming a jelly-like mass. The time interval between the collection and clot formation is called "clotting time".

Under the sterile condition, a deep prick in the fingertip was made. A non-heparinized capillary tube was applied on the pricked fingertip at 45° and the time noted when the blood first appeared in the tube. Capillary action easily filled off the tube, thereafter a small length, about 1 – 2cm from the end of the tube was snapped at intervals of 30 seconds until the formation of fibrin thread across the gap between the ends of the tube. The clotting time of each subject was calculated using the formula: Number of broken

pieces x 30 seconds. The time was recorded in seconds.

Statistical Analysis

The data were expressed as mean $\pm$ standard deviation and statistical analyses were performed using IBM SPSS version 20.0 and excel 2013 for windows. Statistical comparisons of measured variables were carried out using students' t-test and Pearson's correlation was used to find out any correlation between variables. Values were considered statistically significant at $p < 0.05$.

Results

The study recruited 120 female secondary school students who were in the menstrual phase as the study group while 69 who were not in menstrual phase were recruited from the same cohort as controls. The mean age of the study group was 16.48 ± 1.13 while that of the control group was 16.74 ± 1.22 . There was no statistically significant difference in the age of the subjects in both groups ($p = 0.15$). The average ages at menarche for both study and control groups were 12.38 ± 0.94 , and 12.93 ± 1.46 , respectively. The difference was statistically significant ($p = 0.01$). The study group had statistically significant shorter bleeding time compared to the control group (129.63 ± 47.74 and 234.78 ± 38.49 , respectively, $p = 0.01$). This implies higher platelet activity in the menstrual phase compared to other phases. Similarly, the study group had statistically significant shorter clotting time compared to the controls (175.67 ± 42.12 and 404.06 ± 60 , respectively, $p = 0.01$). This implies increased thrombotic and fibrinolytic activities during menstrual phase compared to other phases. The results of age, bleeding and clotting times of the subjects are shown in table 1.

About 50% of the subjects in the study group used 2 pads per day while 42% used at least 3 pads per day. Those that used 1 pad per day had significantly ($p = 0.01$) longer clotting time (240.00 ± 00) compared to those that used 2 pads (205.15 ± 24.1) or at least 3 pads (135.10 ± 22.4) per day. There was no statistically significant difference in bleeding time among the study subjects based on the number of pads used per day ($p = 0.24$) – Table 2.

As the number of pads used per day increases both bleeding time ($r = -0.54$, $p < 0.05$) and clotting time ($r = -0.82$, $p < 0.05$) decrease = Table 3. About 76% of the subjects had a menstrual flow duration of 4 – 7 days, 20% had a flow of 1 – 3 days while only 4% had a flow duration of more than 7

days – Figure 1.

More than 50% of the subjects use 2 – 3 pads per day implying moderate menstrual flow while about 18% use 1 – 2 pads per day implying scanty flow Figure 2.

Table 1: Age, bleeding, and clotting time of the study and the control groups.

	Control group (n= 69)	Study group (n= 120)	t-value	p-value
Age (year)	16.74 ± 1.22	16.48 ± 1.13	1.42	0.15
Age at menarche (year)	12.93 ± 1.46	12.38 ± 0.94	2.78	0.01*
Bleeding Time (BT) (sec)	234.78 ± 38.49	129.63 ± 47.74	16.53	0.01*
Clotting Time (CT) (sec)	404.06 ± 60	175.67 ± 42.12	27.64	0.01*

*Statistically significant association

Table 2: Comparison between a number of pads used per day and the BT and CT among the study subjects.

Parameters	1 pad/day (n=1)	2 pads/day (n=68)	≥ 3 pads /day (n=51)	F	P
BT (sec)	120.00 ± 0 .0	136.10 ± 52.1	121.18 ± 40.7	1.5	0.24
CT (sec)	240.00 ± 0.0	205.15 ± 24.1	135.10 ± 22.4	134.6	0.01*

*Statistically significant variable

BT = Bleeding time

CT = Clotting time

Table 3: Correlation between the number of pads used per day and BT and CT among the study subjects

Variable	R	p-value
BT	-0.54	<0.05
CT	-0.82	<0.05

r = Pearson's correlation coefficient

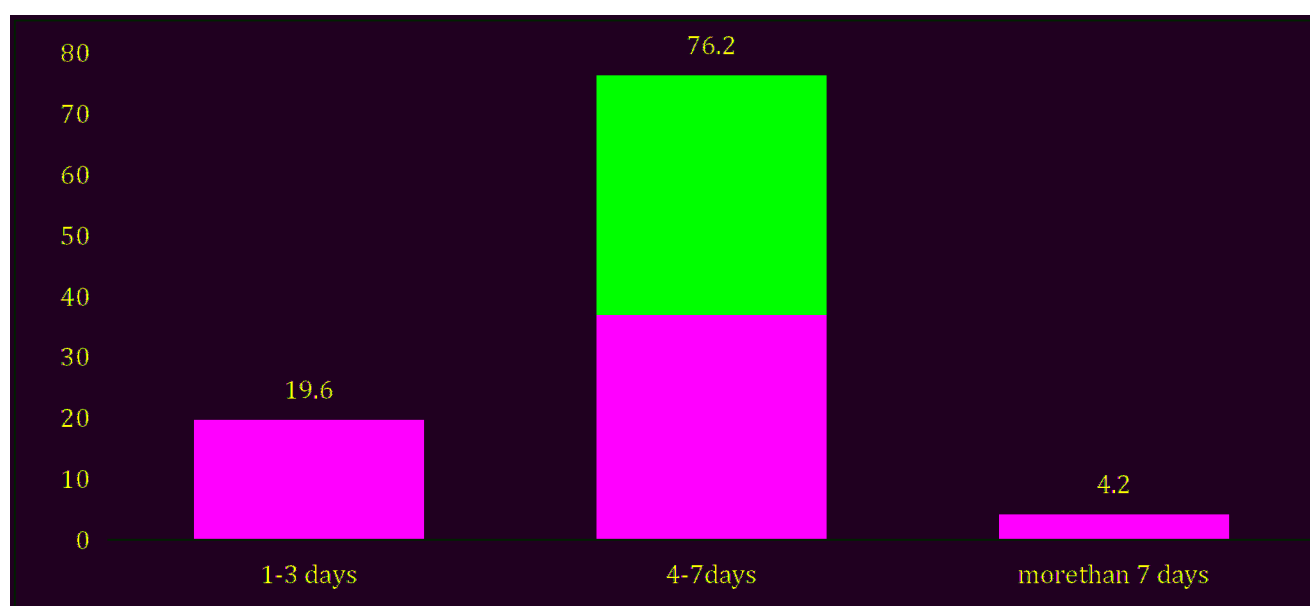


Fig. 1: Duration of the menstrual phase of the respondents.

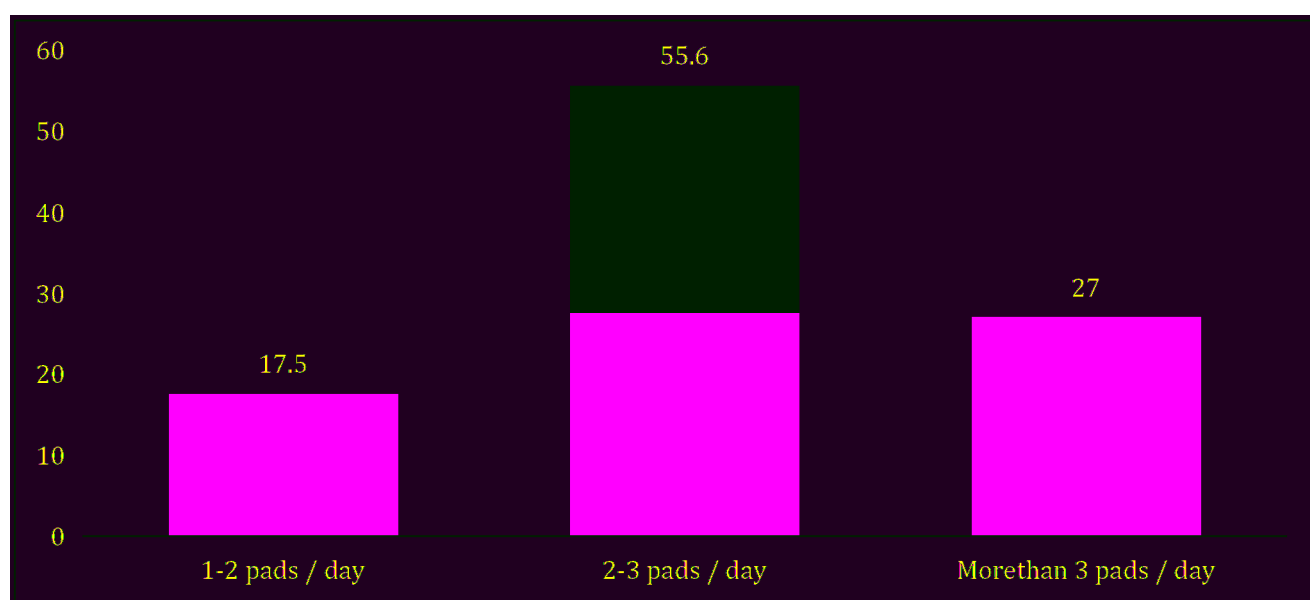


Fig. 2: Menstrual flow of the respondents.

Key:

1 pad = Light

2 pads = Moderate

3 and more pads = Heavy.

Discussion

Menstruation is a regular cyclic change taking place every month within a female reproductive life. It

normally starts at menarche, which is the period when the first endometrial sloughing commences. In this study, both study and control groups attended

the menarche before the age of 13 years. However, the study group had a statistically significant lower age at menarche compared to the controls. The age at menarche for both groups is similar to the 12.83 years reported by Garba *et al.* [7] among adolescent girls in Kano, northwest Nigeria but slightly lower than what was documented elsewhere; ([8, 9]) both of which documented the mean age of menarche in Ibadan school girls to be 13.8 years while Acharya *et al.* [10] and Nair *et al.* [11] recorded 13.3 years and 13.6 years in different parts of India respectively. In South Africa, Henneberg and Loun [12] obtained 13.3 years, while in Sokoto, Northwest Nigeria, Tunua *et al.* [13] documented 15.3 years among their cohorts. This shows that the age at menarche differs along ethnic and racial lines. Obesity, a growing global problem among adolescents, has been associated with lower age at menarche.[7] Other factors that could possibly explain the variation in age at menarche between the two groups include lifestyle modification in relation to food consumed by these cohorts. However, this study did not assess anthropometric parameters and nutritional status of the two groups at menarche and hence it is difficult to draw a conclusion.

Up to 76.2% of the subjects had an average menstrual flow duration lying between 4-7 days. Other workers observed variability in the duration of the menstrual flow; Karthiga *et al.* [14], documented duration of 1 to 5 days, while, Lawrence [15] documented 2 to 7 days. This shows that normal menstrual duration has a wide margin and is independent of the ethnic background of the females involved.

Menstrual blood loss was assessed conservatively by documenting the number of sanitary pads utilized per day as reported verbally by the respondents. Up to 56.7% used two pads in a day, while 42.5 used three pads and above per day and only 0.8% used only one pad in a day as depicted in figure 2. It is possible that females use more sanitary pads during heavy menstrual flow compared to the light one. Nevertheless, studies have clearly shown that the most important determinants for the number of sanitary products used were the woman's personal hygiene practices, frequency of attention to menstrual flow and financial resources.[16, 17] Those who used only two sanitary pads per day could be

having moderate menstrual blood flow, but those who used three pads and above may point towards having menorrhagia if personal hygiene was not taken into account. Those who used only one sanitary pad per day might be at the extreme of the menstrual phase during which the menstrual blood flow is scanty or they were in their anovulatory cycle stage because there is an association between lighter bleeding and anovulation which was noted in several observational studies among perimenopausal women. [18, 19] It was also observed that the menstrual blood loss after ovulatory cycles was far greater than blood loss after anovulatory cycles. Another study reported that women with heavy menstruation were more likely to have had an ovulatory cycle compared with anovulatory one.[20] It is important to note that the use of sanitary pad in quantification of menstrual blood flow can not be utilised as an objective method of grading the severity of menstrual blood loss, but can be used just as a guide because of wide variability in their absorbance rate and brand types which lack standardisation to achieve a common measurement.

Bleeding and clotting times during the menstrual phase were compared between the study and control groups. There was a significant ($p < 0.05$) decrease in bleeding and clotting times in the study subjects as opposed to those in controls. The decrease in the mean values of both bleeding and clotting times was believed to be due to changes in blood coagulation and fibrinolytic processes occurring during menstruation that create a situation of hypercoagulability. This phenomenon could be due to hormonal changes, which invariably protect the woman from life-threatening bleeding during menstruation.

After ovulation, a rise in progesterone levels stimulates secretory changes in the endometrium that could be attributed to ovulation itself.[21] The endometrium achieves full maturity after these morphologic changes and may be thicker. It is possible that these postovulatory changes in the endometrium could result in a higher amount of menstrual blood loss than if the woman was anovulatory, thus giving insight into the biologic mechanism of the effect of ovulation and progesterone on menstrual bleeding.[22]

Sulaxane *et al.*^[23] studied the changes in BT and CT in various phases of the menstrual cycle among 60 healthy females. It was observed that BT at follicular phase was significantly lower than menstrual and luteal phases. Among all the phases of menstrual cycle, follicular phase showed lowest mean of CT, which was not statistically significant. This study did not assess the BT and CT during the follicular phase of the cycle but some workers; Prajapati *et al.*^[24] suggested that hormonal fluctuation during the menstrual cycle does affect the coagulation cascade by producing parallel changes in the prothrombotic tendency and the fibrinolytic activity of healthy women. There is variation in the number of platelets and platelet retention during different phases of menstrual cycle.^[25] The variations in the levels of platelet during different phases of menstrual cycle may be explained by variations in the levels of ovarian hormones, menotoxin (toxic reaction) and toxic resorption of necrotic materials from raw endometrial surface or due to an endocrine reaction via the spleen on haemopoietic system or due to corticosteroids.^[26] However, contrary to the above findings some studies have reported mild changes in platelet count, but no statistically significant changes were observed during different phases of menstrual cycle.^[27]

Balasubramaniam *et al.*^[28] concluded that increase in platelet count could be the valid reason for shortening of the BT during the mid-cycle, they found that mean BT at ovulatory phase or mid-cycle was significantly lower in comparison to other phases of menstrual cycle, and that could be due to the combined effects of higher availability of platelets and aggregation around the pre-ovulatory peak of estrogen. The initial 4 days of menses appeared to have lower platelet count which then starts to raise afterward. However, platelet count may be raised by $1,400 \times 10^9/L$ in blood at the time of ovulation. The platelets count was not assessed in this study, hence we cannot comment on the putative role of platelets on the decline in both the BT and CT in our study.

Rajnee *et al.*^[29] studied the effect of the menstrual cycle on hematological parameters among 30 healthy females in the age group of 18–23 years. The

CT was taken on the 2nd, 11th, 14th, and 22nd days of menstrual cycle. It was found that the CT did not significantly vary during all the phases of menstrual cycle. CT was at its least value around the mid-cycle, and it increases during secretory phase. Angel *et al.*^[30] studied the variation in bleeding and clotting time during the menstrual cycle on forty healthy females (mean age 21 ± 2), there was no significant variation in CT observed during all the phases of menstrual cycle. It seems, therefore, that there are variations in the mean values of BT and CT across the different phases of the menstrual cycle and these variations could be explained on the basis of different protocols employed in assessing these variables.

Correlation analysis revealed an inverse relationship between the number of sanitary pad used per day and bleeding and clotting times. This shows that those who used one pad per day have higher values of bleeding and clotting times compared to those using more than two pads. It also indicates that the use of a single pad is more frequent at the extreme of the menstrual phase. Those who use two to three pads per day have correspondingly lower bleeding and clotting times, supporting the tendency to hypercoagulability state.

Conclusion

Adolescent girls in their menstrual phase have statistically significant shorter bleeding and clotting times compared to those in other phases of the menstrual cycle. Further studies should include the platelets counts in order to fully comment on the patten of bleeding and clotting times during the menstrual cycle.

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Conflict of Interest The authors declared that there is no conflict of interest.

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Chemical Profiling of Gasca N Herbal Formulation using Gc-Ms Revealed Thiazole, 3-Aminopropionitrile, N-(3-Methylbutyl) Acetamide and Hydrazine as Bioactive Compounds

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Abstract

Detailed knowledge of the chemical components of Gasca N; a herbal medicinal product developed from the extracts of *Hyphaene thebaica* and *Adansonia digitata* mixed with *Gum Acacia*, is a potential step in the development of effective therapy against cancer. In the present study, gas chromatography coupled mass spectrometry (GC-MS) was employed to identify the most bioactive chemical compounds in Gasca N herbal product. In total 19 compounds were identified with potential anticancer activity. The results revealed the presence of thiazole, 3-aminopropionitrile, urea, N-(3-methylbutyl) acetamide, 2,4-Pentadienenitrile, methylene cyclohexanol, 2-butanamine, 4-cyclopentene-1,3-diol, Hydrazine, and Carbonyl group. By taking advantage of the chemical knowledge from Gasca N herbal product, numerous therapeutic agents can be developed to target enzymes and protein in cancer metabolism. This will provide a backbone knowledge for designing new anti-cancer compounds using a natural product as a template.

Keywords: Cancer, Gasca N, Herbal therapy, Acetamide, Thiazole

Introduction

With the rapid rise in cancer, a large volume of information is generated on the pharmacological effects, usage, and the development of plant-based herbal medicine as anti-cancer and cancer prevention agents. For many centuries plants have been the main source of crude drugs used to cure or alleviate human sickness. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives.

For example, Thiazoles are novel heterocyclic compounds containing sulphur and nitrogen atoms present in many anticancer drugs currently in use. Thiazoles and their derivatives have received great application as antineoplastic agents after the discovery of the thiazole-based cytotoxic agents tiazofurin.^[1-3] Several studies of the anticancer activity of certain thiazole analogs have been reported by medicinal chemists.^[4-10] Thiazole

derivatives anastrozole and letrozole are the aromatase inhibitors, currently finding widespread application in the clinic as the gold standard for breast cancer treatment.^[11]

Given the wealth of plants derived chemotherapeutic agents, plant bioactive compounds will be invaluable and a useful source of new cancer medication with less toxicity from the natural origin. Although herbal medicines are being used worldwide, their safety and efficacies are still being studied traditionally. It has been reported that between 9 and 81% of cancer patients use at least one type of complementary or alternative therapy after cancer diagnosis^[12-13] to prevent or relieve some of the symptoms of cancer itself.^[14] Self-medication with herbal medicines and other 'natural' substances is a common practice among many cancer patients in Africa to help alleviate some of the side effects from cancer treatments, such as radiotherapy or

chemotherapy.^[15] They use it to treat any associated condition, such as anxiety or depression, or to provide a sense of control or a feeling of active involvement in cancer treatment^[16] The activity of most plants against diseases is due to the bioactive compounds found within the plants. Bioactive compounds present in plants provide therapeutic value through a synergistic effect. Potent plant-derived compounds that are currently in use as cancer drugs are vinblastine, vincristine and camptothecin derivatives which are used in combination with other drugs against different cancer types^[17] Approximately 74% of all anti-cancer agents that reached the market between 1981 and 2002 were plant compounds or semi-synthetic analogues based on plant compounds.^[18]

Gasca N is a locally produced traditional medication for the management of cancer developed from *Hyphaene thebaica* extract as the major ingredients which is a desert palm native to Egypt, sub-Saharan Africa, and West India. It is known as the Doum or gingerbread. The dried fruit pulp is widely consumed as a health tonic, for its many anecdotal medicinal properties, for centuries. ^[19] Antioxidant and anticancer activities of doum have been reported in the aqueous extract of the mesocarp; this is due to the substantial amount of their water-soluble phenolic contents. ^[20,21] The presence of phytochemical called lignans, could well be responsible for the anti-carcinogenic action as mentioned by Carter.^[22] Compounds identified using thin-layer chromatography showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils, and flavonoids, at the same time the fruit has been shown to lower blood pressure in animal models. ^[20]

In this perspective, the present study aims to evaluate the bioactive components with potential anti-cancer activity in Gasca N herbal medicine used for the management of cancer. The study will attempt to link the acclaimed therapeutic indication of Gasca N in the management of cancer with possible bioactive compounds. Given the potential risks and lack of sufficient evidence and knowledge of the constituents and use of most herbal medicines by cancer patients in Africa, it has become necessary

to investigate these medicinal products to understand better the bioactive components they are made up of. This is an essential first step in the development of herbal medicinal products with less toxicity and potential activity.

Materials and Methods

Materials

Gasca N sample (*Hyphaene thebaica*) was obtained directly from the manufacturer at Greenleaf herbal product situated at no 5664 Yan dodo, Hotoro, Nassarawa Local Government area of Kano State. All chemicals were of the analytical reagent grade.

Chemicals

HPLC grade methanol.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The Gasca D herbal formulation powdered sample was dissolved in HPLC grade methanol to appropriate concentrations for GC-MS analysis. The sample was analysed by GC-MS on an Agilent 6890N series gas chromatography coupled with a LECO time of flight mass spectrometer detector (MS-TOF; Agilent Technology, Santa Clara, CA) with ionization voltage of 70 eV and equipped with fused silica non-polar DB-5 capillary column (10m x 0.18 mm; film thickness 0.18 mm; Agilent HP). The operating conditions were as follows: the injector operated in split mode (ratio 20:1) and mass spectrometer transfer line temperatures were set at 200 and 300 °C, respectively; the oven temperature gradually rose from 40 to 260 °C, at warming rate of 4 °C min⁻¹, kept at 260 °C for 20 min, and finally up to 340 °C, at a warming rate of 4 °C min⁻¹ for 20 min isothermally; the injector temperature was set at 300 °C. The carrier gas (He) was set to 1mL min⁻¹ flow. The mass analyser was used in full scan mode scanning from m/z 40-550 and mass spectra were taken at 70 eV. Compounds were identified by co-injection of the sample with standard references by comparison of their mass spectral data with those of NIST Mass Spectral Library 98 and equipment Libraries as well as by comparison of their retention indices (RIs).

Results

The bioactive compounds with their, concentration (peak area %), and retention time (RT) are presented in Table 1, and the chemical structure of the identified components shown in Figure 1. The GC-MS analysis revealed the presence of biologically

important active compounds with potential anticancer effects including Thiazole, 3-Aminopropionitrile, 2,4-Pentadienenitrile, 2,3-Diamino-2-cyanosuccinonitrile, N-(3-Methylbutyl) Acetamide and Hydrazine, etc.

Table 1: Bioactive compounds identified in Gasca N herbal formulation using GC-MS analysis.

S/N	RT(min)	Peak Area %	Compound(s)	Mol. formula	M.wt (g/mol)
1	50.8, 53.9	1,2	Urea	CH ₄ N ₂ O	60.6
2	53.9, 60.1, 63.9, 67.0, 71.1	2,5,10,13,18	Hydrazine	H ₄ N ₂	32.05
3	57.2, 60.1, 61.3	3,5,6	2-Butanamine	C ₄ H ₁₁ N	73.14
4	58.9	4	3-Aminopropionitrile	C ₃ H ₆ N ₂	70.09
5	61.3, 67.0, 71.1	6,13,18	Carbonyl sulfide	COS	60.08
6	62.3	7	2,4-Pentadienenitrile	C ₅ H ₅ N	79.10
7	62.7	8	Isobutyl amine	C ₄ H ₁₁ N	73.14
8	63.3, 71.1	9,18	Formic acid hydrazide	CH ₄ N ₂ O	60.06
9	68.6	15	Cyclopropane ethanol	C ₅ H ₁₂ O	88.15
10	69.6, 77.0	16,28,37	Cyclobutanone, Methylene cyclohexanol	C ₄ H ₆ O C ₇ H ₁₂ O	70.09 112.17
11	70.4	17	2-Propanamine	C ₃ H ₉ N	59.11
12	71.4, 73.2, 75.2, 75.6, 78.1, 78.5, 80.9	19,22,26,27,31	Carbonic acid, Thiazole, 4-Pentenoic acid	CH ₂ O ₃ , C ₃ H ₃ NS, C ₅ H ₈ O ₂	62.02, 85.13, 100.12
13	71.9	20	Cyclopropane, 2,3-Diamino-2-cyanosuccinonitrile	C ₃ H ₆ , C ₅ H ₅ N ₅	42.08, 135.13
14	79.9	35	4-Cyclopentene-1,3-diol	C ₅ H ₈ O ₂	100.12
15	80.5, 90.1	36,48	N-(3-Methylbutyl) acetamide	C ₇ H ₁₅ NO	129.20

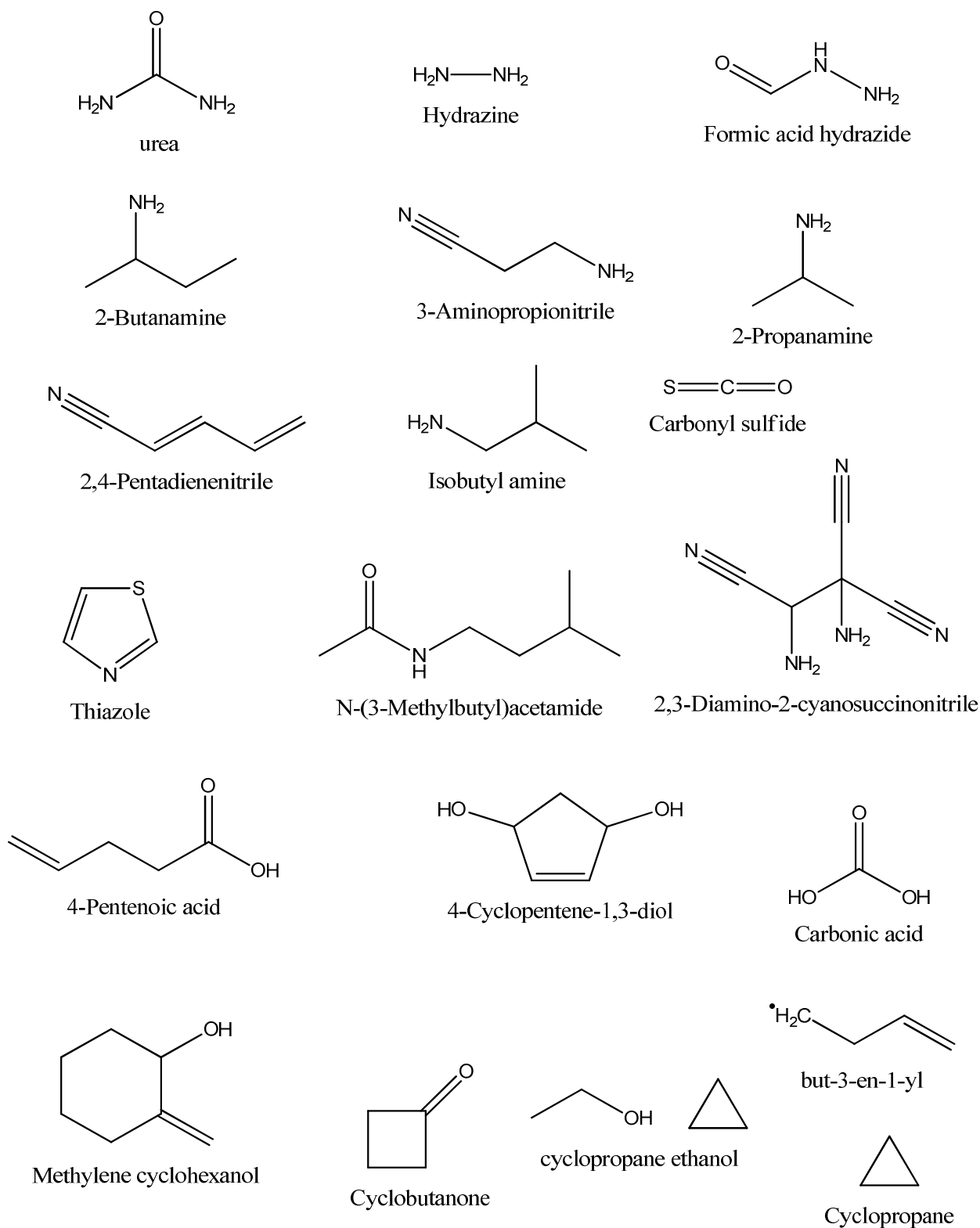


Fig. 1: Chemical structures of compounds identified in Gasca N using GC-MS.

Discussion

Various classes of secondary metabolites were identified in Gasca N herbal product which could thus be used for the management of various health conditions associated with the metabolites identified. Many herbal medicines have been reported to have a similar functional bioactive component with advanced conventional medication, this similarity may account for their potency in similar target groups.^[23] Among the compounds identified are the Thiazoles which is a common structural motif in a large number of anti-cancer agents.^[24-29] Marianna et al.^[31] reported that thiazole compounds could suppress tumor growth in a human breast cancer xenograft model and may have potential anticancer activities against breast cancer *in vivo*. Millennium Pharmaceuticals, Inc.^[32] claimed an invention concerning the preparation of thiazole derivatives that inhibits PI3K. It has been reported that compounds which are inhibitors of the activity of PI3Ks may be useful in the treatment of disorders such as respiratory diseases, allergic diseases, cardiovascular diseases, hematologic malignancies, cystic fibrosis, neurodegenerative diseases, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, cancer, sperm motility, transplantation rejection, graft rejection among others.^[33] The presence of thiazole derivative in Gasca N herbal product may contribute to the therapeutic potential of Gasca N herbal product in the management of cancer.

Interestingly, 3-Aminopropionitrile has been identified in Gasca N herbal product, which has a structural similarity with β -Aminopropionitrile (BAPN). β -Aminopropionitrile (BAPN) is an active site inhibitor of Lysyl oxidase (LOX), a hypoxia-responsive gene that has been shown to be essential for hypoxia-induced metastasis in breast cancer. Treatment of tumor cells with β -aminopropionitrile (BAPN), blocked the hypoxia-induced epithelial-mesenchymal transition (EMT) morphological and marker protein changes and inhibited invasion and migration capacities of cervical carcinoma cells *in vitro*.^[34] BAPN was also found to reduce the development of bone metastases and significantly decrease the frequency of metastases in animals when challenged by the introduction of a large

number of breast cancer cells directly into the arterial circulation.^[35]

The use of urea in the treatment for certain types of cancers is of great interest. Yingzhuan et al.,^[36] showed that biphenyl urea derivate TPD7 (*N*-(4'-acetyl-3',5,6-trimethoxybiphenyl-3-yl)-*N'*-[4-(3-morpholin-4-ylpropoxy)phenyl]urea) exerted its anti-invasive effect through the down-regulation of CXCR4 (chemokine receptor 4) expression and thus had the potential for the treatment of breast cancer. Urea compound was found to be present in Gasca N herbal product, suggesting a possible link between Gasca N and good treatment outcome. Danopoulos and Danopoulou^[37] had reported the anticancer activity of urea where they applied urea as a chemotherapeutic agent in the treatment of skin malignancies and cancer of the liver. Donopoulos and Donopoulou,^[37,38] also reported that oral urea was useful in producing regression of liver cancer in patients. Gandhi et al.,^[39] reported the use of oral urea treatment in two cases of adenocarcinomatous multiple secondaries in the liver of patients who showed marked reduction in the size of the liver, itching was less intense, appetite improved, and the patients gained an average of 1.81 kg, over a period of 2 months. These patients were alive at the end of 3.5 months.

The presence of acetamide has been found in Gasca N herbal product. Acetamide derivatives are a new class of potential chemotherapeutic agents that have been found to exhibit varying degrees of potency and selectivity *in vitro* against LNCaP (androgen-sensitive human prostate adenocarcinoma cells). Barrett et al.,^[40] reported the cytotoxic effects of the acetamide drugs on prostate cancer cells following 10 μ M continuous drug exposure for 24 h. An anticancer drug marketed by the name Mekinist composed of Trametinib as the active molecule is a derivative of acetamide, *N*-(3-{3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2*H*)-yl}phenyl)acetamide. Another derivative of Acetamide 2-{2-[3-(benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-4-chlorophenoxy}-*N*-(4-methoxyphenyl)acetamide was found to be effective against tumor.^[41]

The GC-MS analysis of Gasca N herbal product revealed the presence of hydrazine. Derivatives of hydrazine, such as hydrazine sulfate have been found to be capable of inducing a wide range of therapeutic effects in late-stage cancer patients. Hydrazine sulfate was reported to yield up to 70% anticachexia and 41 % antitumor response, in long-term studies involving as many as 84 and 95 evaluable patients, respectively^[42-46].

Conclusion

The identification of bioactive compounds with potential anticancer activity in Gasca N herbal formulation can help in designing new therapeutic

compounds with potent activity against cancer. Further investigations are required to isolate and elucidate the structure of the functional bioactive compounds and further screen their pharmacological activity.

Conflict of Interest

There is no conflict of interest.

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Effect of Oral Administration of Aqueous Extract of *Tamarindus indica* Pulp on Diabetes Mellitus in Rats

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Abstract

The aim of this study is to assess hyperglycaemic activity of the aqueous fruit pulp extract of *Tamarindus indica* (AFPETI) in alloxan-induced diabetic rats. A total of forty-eight rats were used for testing the anti-diabetic effect of AFPETI and were grouped into six of eight rats each. Group I was used as normal control, groups II-VI were induced with diabetes using alloxan monohydrate (150mg/kg body weight). Group III, IV, and V were daily administered with 200, 400 and 600mg/kg body weight respectively of AFPETI while group VI was daily administered with standard diabetes drug chlorpropamide (84mg/kg) for 2 weeks. The effect of the extract on fasting blood glucose (FBG) level and total protein (TP) were determined weekly. All rats in the diabetic groups had FBG levels within the diabetic range at the initial stage of the experiment and the fasting blood glucose level significantly ($p < 0.05$) dropped in dose-dependent manner after two weeks of treatment, with 600mg/kg dosage being most significant ($p < 0.05$). The serum total protein increased, after treatment with AFPETI, which was significant ($p < 0.05$) when compared with the diabetic control group II after two weeks. However, the glucose-lowering effect of AFPETI is less than the standard diabetic drug (chlorpropamide). The results suggest that AFPETI may ameliorate diabetes mellitus in rats, although it is not statistically significant when compared with the standard diabetic drug chlorpropamide

Keywords: *Tamarindus indica*, hyperglycaemia, Fasting blood glucose, Chlorpropamide, Diabetes.

Introduction

The rapid increase in the number of people with diabetes mellitus worldwide is quite alarming. [1] At least, 177 million people worldwide live with diabetes and this figure is likely to increase by 2030. [2] The International Diabetes Federation (IDF) estimated that 10.8 million people have diabetes in sub-Saharan Africa in 2006 and that this would rise to 18.7 million by 2025 [3]. In Nigeria, about 3% of the adult were reported to have diabetes mellitus. [4]

It has been reported that diabetic patients have significant defects of antioxidant protections and generation of reactive oxygen species (oxidative stress) which may play an important role in the etiology of diabetic complications. [5] Hyperglycaemia is known to play a pathogenic role due to the excess formation of glucose-derived end products. [6] Diabetes mellitus, an endocrine and metabolic disorder characterised by chronic hyperglycaemia produces multiple biochemical impairments and oxidative stress especially an increased susceptibility to lipid peroxidation that

plays role in the progression of the symptoms of diabetes. [7] Several hypotheses have been postulated to explain the development of free radicals in diabetes which include auto-oxidation of glucose, enzymatic and non-enzymatic glycation of proteins with increased formation of glucose-derived advanced glycosylation end products, enhanced glucose flux through polyol pathway [8] and reduction of anti-oxidant defence. [9]

Medicinal plants are the backbone of traditional medicine [10]. Medicinal plants have been used in various systems, as they have potential against numerous diseases. Every part of the plant from root to leaf tips is useful for human needs. Contrary to pharmaceuticals, it is often freely and readily available. [11] Costs are significantly low; herbs have usually little or no toxicity during long-term oral administration and are relatively available at large scale. [12]

Tamarindus indica Linn is a tree-type of plant belonging to the Caesalpiniaceae family [13] grows

naturally in tropical and subtropical regions and now is one of the most important plant resources as food materials and is accepted as herbal medicine in parts of the world. ^[14] *T. indica* is rich in nutrients and plays an important role in human nutrition, mainly in developing countries. *T. indica* Linn was used as a traditional medicine for the management of diabetes mellitus in human and experimental animals. ^[13,15,16,17]

Many of the chronic complications of diabetes involve changes in structural proteins. It is thus possible that changes in protein metabolism are responsible for many of the chronic complications of diabetes mellitus because even a minor imbalance between protein synthesis and degradation can potentially have a profound effect over the long term on cell viability and metabolism. Alterations in protein synthesis and degradation can also adversely affect the repair of tissue after injury or infection. The capacity of nutrients to stimulate insulin release from the pancreatic β -cell reflects their capacity to augment oxidative fluxes in the islet cells ^[18]. The American Diabetes Association recommends a post-meal glucose level of less than 10 mmol/L (180 mg/dL) and a fasting plasma glucose of 5 to 7.2 mmol/L (90–130 mg/dL). ^[19]

This study was undertaken to evaluate the antihyperglycaemic effect of the aqueous extract of *Tamarindus indica* fruit pulp in alloxan-induced diabetic rats as a way of validating its traditional usage. Modern synthetic antihyperglycaemic agents were reported to produce undesirable and unwanted side effects such as; weight gain, lactic acidosis, and hypoglycaemia and are not easily or cheaply available. Thus, more alternative therapy and approach with less or no side effects are needed to manage diabetes mellitus and its complications.

Materials and Methods

The chemical and reagents used for this study are of highest quality obtainable and these includes: Alloxan Monohydrate (Explicit Chemicals, PGT India), Glucose (Gluc-pap) Kit-Randox Laboratories Ltd United Kingdom, Total protein (TP) Kit-Randox Laboratories Ltd United Kingdom, Chlorpropamide (Diabenese) Neimeth International Pharmacy-Oregon, Lagos, Nigeria, Others include Chemical

reagents (such as normal saline, biuret reagent) and distilled water.

Sample Collection and Preparation of Extract

Fresh matured Tamarind fruits were obtained from Rimi Market in Kano city, Kano State, and was kept away from direct sunlight and allowed to dry. The dried fruits were peeled and the pulps were separated from the seed. The aqueous fruit pulp extract was prepared by soaking 100g of the pulp in one litre of distilled water and mixed thoroughly (0.1g/mg; 100mg/ml). After mixing, the extract was refrigerated without evaporation. The volume (cm³) of the pulp aqueous extract given to each rat was determined by its weight and required dose as follows:

$$\frac{\text{Volume administered (cm}^3\text{)} = \text{weight of rat (kg)} \times \text{required dose (mg/kg)}}{\text{Concentration of the extract (mg/cm}^3\text{)}}$$

Experimental Animals and Induction of Diabetes

Albino rats were obtained from the Animal House of the Biological Sciences Department, Bayero University, Kano. The rats were maintained under standard laboratory conditions and were allowed free access to both food and water throughout the period of the experiment.

Alloxan solution was prepared by dissolving alloxan monohydrate (0.9g) in distilled water (6cm³) and diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150mg/kg). The volume of the solution containing 150mg/kg given to each rat was determined by its weight according to the following relation:

$$\frac{\text{Volume administered (cm}^3\text{)} = \text{weight of rat (kg)} \times 150(\text{mg/kg})}{\text{Concentration of the solution (150mg/cm}^3\text{)}}$$

The group of rats to be induced was divided into six groups; the rats in Groups II, III, IV, V, and VI were intraperitoneally administered with alloxan solutions respectively as determined by their body weight.

Experimental Design

Forty-eight rats were divided into six groups of eight rats each; group I was used as normal control,

groups II - VI were induced with diabetes by intraperitoneal administration of alloxan monohydrate (150mg/kg) and was used as indicated below.

Group 1: Normal control rats. (Non-diabetic rats and was not administered with aqueous extract (0.0mg/kg) *T. indica* fruit pulp.

Group 2: Diabetic control rats. (Diabetic rats not administered with aqueous extract (0.0mg/kg) *T. indica* fruit pulp.

Group 3: Diabetic rats orally administered with aqueous extract (200 mg/kg) of *T. indica* fruit pulp.

Group 4: Diabetic rats orally administered with aqueous extract (400 mg/kg) of *T. indica* fruit pulp.

Group 5: Diabetic rats orally administered with aqueous extract (600 mg/kg) of *T. indica* fruit pulp.

Group 6: Diabetic rats orally administered with chlorpropamide (standard drug) (84 mg/kg).

The blood glucose level of the rats was determined before and after the induction of diabetes using a glucometer. Rats with blood glucose levels above 180mg/dl after induction were considered diabetic and were selected for the study. After the 7th and 14th dose of treatment with 200, 400, and 600mg/kg of aqueous extract of *T. indica* fruit pulp or 84mg/kg of chlorpropamide, four of the rats were sacrificed from each group. Blood samples were collected for estimation of serum glucose and total protein.

Serum Glucose and Protein Determination

Serum glucose was determined using the method of Barham and Trinder. [20] Serum total protein was determined by Biuret method. [21]

Statistical Analysis

Results were expressed as mean±standard deviation and analysed by analysis of variance (ANOVA) and differences were considered statistically significant at $p < 0.05$.

Results

Table 1: Effect of aqueous fruit pulp extract of *Tamarindus indica* on blood glucose concentration and serum total protein levels of alloxan-induced diabetic rats after 7 days.

Groups	Serum Glucose (mmol/l)	Serum Protein (g/dl)
Normal Control	4.92±0.61*	7.63±0.20*
Diabetic Control	12.42±1.14*	5.00±0.01*
Diabetic Rats + 200mg/kg AFPETI	12.11±0.26	5.07±0.09
Diabetic Rats + 400mg/kg AFPETI	11.82±0.24	5.15±0.06
Diabetic Rats + 600mg/kg AFPETI	11.38±0.30	5.28±0.23
Diabetic Rats + 84mg/kg Chlorpropamide	9.97±0.05 ^a	6.25±0.23 ^a

Results are mean ± standard deviation. Values with the same superscripts* in a column are significantly different with respect to each other ($P < 0.05$). Values with different alphabetical superscript ^a in a column are significantly different with respect to the diabetic control ($P < 0.05$).

AFPETI: Aqueous fruit pulp extract of *T. indica*

Table 2: Effect of *Tamarindus indica* aqueous pulp extract on blood glucose concentration and serum total protein levels of alloxan-induced diabetic rats after 14 days.

Groups	Serum Glucose ((mmol/l)	Serum Protein (g/dl)
Normal Control	4.77±0.42*	8.07±0.26*
Diabetic Control	12.11±0.55*	5.05±0.13*
Diabetic Rats + 200mg/kg AFPETI	11.69±0.22	5.29±0.15
Diabetic Rats + 400mg/kg AFPETI	11.18±0.27 ^a	5.56±0.35 ^a
Diabetic Rats + 600mg/kg AFPETI	10.89±0.08 ^b	5.69±0.36 ^b
Diabetic Rats + 84mg/kg Chlorpropamide	7.46±0.35 ^c	7.00±0.03 ^c

Results are mean $\pm$ SD; n= 4. Values with the same superscripts in a column are significantly different with respect to each other (P<0.05). Values with the different alphabetical superscript ^a, ^b, ^c in a column are significantly different with respect to diabetic control (P<0.05).

AFPETI: Aqueous fruit pulp extract of *T. indica*.

Discussion

The diabetic control group exhibited an increase in fasting blood glucose during the experiment. The alloxan-induced diabetic rats elicited significant rise in blood glucose to 12.42 mmol/L (P<0.05) compared to normal control (4.92). Alloxan, a β -cytotoxin, induces diabetes mellitus by damaging the insulin-secreting β -cells of the pancreas, resulting in decreased endogenous insulin release. Intraperitoneal administration of alloxan (150 mg/kg body weight) effectively induced diabetes mellitus in normal rats as polydipsia, polyuria and polyphagia compared with normal rats were observed during the experiment. The major clinical significance of diabetes is often indicated by the presence of symptoms such as polydipsia, polyuria, and unexplained weight loss and confirmed by measurement of abnormal hyperglycaemia. [22]

The effect of oral administration of varying doses of aqueous fruit pulp extract of *T. indica* fruit on alloxan-induced diabetic rats is shown in Table 1. A dose-dependent hypoglycemic effect of the fruit extract was observed; however, the results were significant only after the second week at higher doses of 400 and 600 mg extract/kg body weight. Chlorpropamide at a dose of 84mg/kg body weight demonstrated greater potency on decreasing glucose levels in alloxan-induced diabetic rats. It was observed that after 7 days aqueous fruit pulp extract of *T. indica* slightly reduced serum glucose level in the rats but the reduction was not significant. It was evident from the results that aqueous fruit pulp extract of *T. indica* (AFPETI) reduced the fasting blood glucose level in alloxan-induced diabetic rats. The aqueous extracts-treated diabetic groups at 200, 400 and 600 mg/kg recorded a dose-dependent drop in Fasting Blood Glucose after 14 days. Although the glucose-lowering activity was not comparable to the standard drug chlorpropamide even at the highest dose of extracts of fruit tested, nevertheless both extracts at the higher doses of 400 mg extract/kg body weight and 600mg extract/kg body weight significantly lowered blood glucose levels in

diabetic rats when compared to diabetic controls after 14days. Fruits of the plant are known to contain limonene and have been shown to possess hypoglycemic activity [23]. Additionally, it possesses antioxidant properties, which could be beneficial during diabetes. The anti-hyperglycaemic effect of AFPETI could be linked to more than one mechanism. The possible mechanism includes the stimulation of β -cells and subsequent release of insulin and activation of the insulin receptors. The plant's anti-hyperglycaemic action may be by potentiation of pancreatic secretion of insulin.

In light of the results from Table 1, it indicates that the aqueous extract of *T. indica* exhibited significant anti-hyperglycaemic activity in alloxan-induced diabetic rats after 14days. The anti-hyperglycaemic effects of *T. indica* are possibly linked to their antioxidant properties, which could counteract the toxic and pro-oxidant effects of alloxan. Flavonoids, sterols/triterpenoids, alkaloids, and phenolics are known to be bioactive anti-diabetic principles. [24] Flavonoids are known to regenerate the damaged β cells in the alloxan diabetic rats. [25] Phenolics were also found to be effective anti-hyperglycaemic agents. [26]

A decrease in serum total protein was observed in the diabetic control group and this was reported to be due to net increase in protein breakdown rather than a decline in protein synthesis. [27] After oral administration of the *T. indica* pulp extracts, the alteration in protein metabolism was slightly restored. Poorly controlled diabetes is associated with altered body protein metabolism. [28] Insulin-mediated net protein anabolism occurs largely in skeletal muscle; therefore, the catabolic state resulting from insulin deficiency or resistance may lead to muscle wasting. [29] Similarly, decreased synthesis of hepatic plasma proteins (eg, albumin) has been shown with diabetes. [30] Thus, the goal of diabetes management is to improve glycemic control and to provide adequate nutrient intake to help

reduce muscle wasting, morbidity, and the risk of mortality. ^[31]

Glucose itself and hyperglycaemia increase protein glycosylation which is an important source of free radicals. Elevated glucose causes significant non-enzymatic glycosylation of proteins in diabetes. ^[32] In diabetes, oxidative stress has been found to be mainly due to increased production of oxygen free radicals and a sharp reduction of antioxidant defenses. ^[33] Cell utilizes oxygen in the making of energy, but that process produces free radicals, toxic products that damage DNA and protein. Under physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of free radical production. ^[34]

The result from Tables 1 and 2 shows that the diabetic control rats had low serum total protein levels compared with the untreated control rats, while the diabetic rats showed significant ($P < 0.05$) increase in total protein level after the treatment. After seven days of AFPETI administration to diabetic rats, there was an increase in the serum total protein level although the increase was not significant. After 14 days of administration of AFPETI to diabetic rats, there was a significant increase in the serum total protein level compared to the diabetic rats. Although the administration of AFPETI caused a significant increase in serum total protein in diabetic rats, Chlorpropamide caused a more significant increase. In diabetes mellitus, a variety of proteins are subjected to non-enzymatic glycation and this is thought to contribute to the long-term complications of the disease ^[35]. In the present study, the level of serum total proteins were found to be decreased in diabetic rats which may be ascribed to (i) decreased amino acid uptake; (ii) greatly decreased concentration of variety of essential amino acids; (iii) increased conversion rate of glucogenic amino acids to carbon dioxide and water; and (iv) reduction in protein synthesis secondary to a decreased amount and availability of

mRNA. ^[36] Insulin deficiency leads to various metabolic aberrations in the animals, namely increase blood glucose, decreased protein content and increased levels of cholesterol and triglyceride. ^[37] Oral administration of aqueous fruit pulp extract of *T. indica* improved total protein concentration in the serum after 14 days at a dose of 600mg/kg body weight.

Conclusion

This research investigated the effect of Aqueous fruit pulp extract of *Tamarindus indica* in rats and found that extract may be useful in the management of diabetes mellitus as there was gradual decrease in the serum glucose level of diabetic rats after 14 days of administration in a dose-dependent manner. All the dosages administered (200mg/kg, 400mg/kg, 600mg/kg) had effect on serum glucose and decreased serum glucose level with 600mg/kg having the most effect. Although the standard diabetic drug chlorpropamide used in this research was more effective in the management of diabetes mellitus in rats, *T. indica* fruit pulp may ameliorate diabetes mellitus and be useful in the management of the disease.

Contribution of Authors

Zinat, S. M. carried out the research and laboratory work and wrote the first draft of the manuscript. Sule, M. S. supervised the research work and made corrections to the manuscript. Alhassan, A. J. co-supervised the research work and made extensive corrections to the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest for this manuscript

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Evaluation of Acute Toxicity and Hypoglycaemic Effect of Methanol Extract Fractions of *Ipomoea asarifolia* Leaves on Alloxan Induced Diabetic Rats

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Abstract

The research work investigated the acute toxicity and hypoglycaemic properties of *Ipomoea asarifolia* leaves in alloxan induced diabetic rats. Sixty-five (65) rats were used in the studies, 14 animals were used for toxicity study, while 45 were grouped into five (n=9). Animals in each group were orally administered with one of crude extract or different solvent fractions. Among each of the groups, animals were subgrouped into three and administered varying concentrations (17mg/kg, 35mg/kg or 70mg/kg body weight) of the respective extract. Diabetic induction was done with single intraperitoneal injection of 150mg/kg body weight alloxan; fasting blood glucose was monitored daily for 5 weeks. Results indicated hypoglycaemic activity in rats treated with fractions F1, F2, and F3 with highest activity recorded in F3 and least activity in F4 and F5 in comparison with diabetic control. The acute toxicity test of the crude fraction indicates slight toxicity.

Keywords: *Ipomoea Asarifolia*, Diabetes Mellitus, Hypoglycaemic Effect, Acute Toxicity

Introduction

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular, the blood vessels and nerves. [1] It has now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 425 million and the disease now kills more people than AIDS. [2]

In recent years, a great scientific research interest for the understanding of the etiopathogenesis of diabetes mellitus and the ultimate development of definitive curative and/or prophylactic options in its management have been accelerated. [3] Drug management of diabetes without associated untoward effect has also remained a challenge for orthodox medical practice. This has necessitated the exploration and screening of medicinal plants with acclaimed therapeutic efficacies in the management of diabetes. [4]

Ipomoea asarifolia belongs to the family convolvulaceae; it is an aquatic plant that is found abundantly in muddy stream banks, freshwater ponds, and lakes. *I. asarifolia* is a trailing vine with milky sap and is commonly consumed in Cameroon, Bangladesh, and other countries as a green leafy vegetable. [5] The leaves contain adequate amount of amino acids like aspartic acid, glycine, and alanine in accordance with the WHO recommended pattern for an ideal dietary protein. [6] This can be compared with conventional food crops such as soybeans or whole egg indicating its potential as food supplement. *I. asarifolia* has also been reported to be one of the richest sources of carotenoids and chlorophyll. [7] An extensive study on the identification of these pigments revealed that it contains 12 components. [8] The medicinal value of *I. asarifolia* was notable from the ancient science of Indian medicine and homeopathy. At that time, extracts of *I. asarifolia* leaves were administered orally to alleviate antioxidant related disorders. [9]

The plant is also used effectively against nosebleed and high blood pressure. [10, 11] Further, its leaf extract can be used to reduce blood sugar levels [9]

and as an antibiotic against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.^[12] The floral buds are used as anthelmintic.^[13] This study is aimed at assessing the hypoglycaemic effect of *I. asarifolia* using alloxan-induced diabetic rat model.

Materials and Methods Laboratory Animals

Sixty-five (65) adult Wistar albino rats weighing between 160-240g were obtained from Department of Biological Sciences, Bayero University, Kano. The animals were kept in cages at room temperature for one week to acclimatize and were allowed access to food and water *ad libitum*. Principles of laboratory animal care guidelines were followed.^[16]

Collection and Preparation of Plant Material

The leaves of *Ipomoea asarifolia* (*Duman Rafi* in Hausa) were collected from Nassarawa Local Government of Kano State, Nigeria between February and March 2013. Identification of the plant was done by a botanist at the Department of Biological Sciences, Bayero University, Kano with voucher no. BUKHAN 152. The leaves were pounded and dried in the shade and then pulverized into a fine powder and stored in a labelled plastic container. Plant Extract Preparation Into 500 cm³ of methanol, 200g of the leaves powder was added, shaken vigorously and left to stand at room temperature for 14 days with intermittent vigorous shaking at intervals of 48 hours. After 14 days speculation, the mixture was filtered using Whatman No 1 filter paper. The extract was obtained and concentrated using rotary evaporator. The extract was then freeze-dried (SB4 Freeze Dryer) giving rise to crude methanol extract labelled F1.

Acute Toxicity Study

LD₅₀ of the crude extract was determined using the method of Lorke.^[15] In the first phase, nine rats weighing 160g were used and divided into three groups of three rats each. The animals were treated with crude extract at doses of 1000, 1500 and 2000mg/kg orally. The rats were monitored for 24 hours for mortality and general behaviour. In the second phase, five rats were used and grouped into five and treated with crude extract at doses of 1600, 1700, 1800, 1900 and 2000mg/kg orally, and monitored for 24 hours. Fractionation of Plant Extracts The crude methanol extract of *I. asarifolia* was extracted between chloroform and distilled

water. The crude extract (5.0g) was dissolved in mixture of chloroform and water in a ratio of 1:1 with vigorous shaking for 15 minutes in a separating funnel; the mixture was allowed to stand for 24 hours for complete separation. The chloroform and aqueous fractions were drained separately, the aqueous fraction was then concentrated and freeze-dried to powder and stored in a container labelled F2. The chloroform fraction was washed in equal volume of chloroform fraction and aqueous NaCl. The mixture was mixed vigorously in a separating funnel and allowed to settle. The chloroform fraction was drained and further washed with distilled water in a ratio of 4:1. The chloroform fraction was collected, concentrated, freeze-dried and stored in a container labelled F4.

The chloroform extract (5.0g) was dissolved in 400ml 10% aqueous methanol, then an equal amount of N-hexane was added. The mixture was shaken vigorously for five minutes and allowed to settle in a separating funnel. The two layers were drained into different containers, concentrated, dried and labelled F3 and the N-hexane fraction labelled F5. Induction of Diabetes Diabetes mellitus was induced by a single intraperitoneal (IP) injection of alloxan 150mg/Kg body weight (0.27g alloxan /10ml of normal saline) after 16 hours of fasting. After a window period of 72 hours, rats with fasting blood glucose levels of 11.1mmol/l were considered diabetic.^[16] Experimental Design A total of fifty-one (51) Wistar albino rats were used for the hypoglycaemic study, 45 rats were placed into five groups of nine rats each. Each group was further made into 3 sub-groups of 3 rats each. Doses were given as 17, 35 and 70mg/kg to each subgroup named R1, R2, and R3. The remaining 6 rats were used as normal and diabetic controls with 3 rats each.

Group I: were treated with crude extract (F1)
Group II: were treated with aqueous extract (F2)
Group III: were treated with methanol extract (F3)
Group IV: were treated with chloroform extract (F4)
Group V: were treated with n-hexane extract (F5)
Group VI: served as normal control Group VII: served as diabetic control

Animals were treated for five weeks with the extract, then two weeks without the extract while the fasting blood glucose was monitored weekly.

Sample Collection

At the end of the experimental period, the animals were sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride and centrifuged at 3000g for 15 minutes in a bench centrifuge; the serum was separated and kept at 4°C.

Serum Glucose Determination

Serum glucose was determined using a glucose oxidase method. [17] Three sets of test tubes were set up and labelled blank, standard and test, phenol reagent (1ml) was added to all the three test tubes. Serum (20µl) was added to the test-tube labelled test (20µl), were added to the tube labelled standard and water (20µl) was added to the tube labelled blank. Colour reagent (1ml) was added to all the three tubes. These were mixed, incubated at 37°C for 20 minutes, removed, cooled and absorbance was read at 520nm. The concentration of glucose was then obtained from the relation: $\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of stand}$

Statistical Analysis

Data obtained were subjected to statistical analysis to determine statistical significance using one way

ANOVA at $P < 0.05$.

Results and Discussion Results

The acute toxicity test of crude methanol fraction of *I. asarifolia* was evaluated in two different phases. In the first phase, toxicity symptoms were absent in all the groups. In the second phase, mortality was observed in the group administered the highest dose. LD₅₀ was calculated as follows:

$$LD_{50} = \sqrt{(D_o \times D_{100})}$$

D_o = Highest dose that gave no mortality.

D₁₀₀ = Lowest dose that gave mortality

$$LD_{50} = \sqrt{2000 \times 1900}$$

$$LD_{50} = 1949\text{mg}$$

The effect of the administration of different extracts of *I. asarifolia* on the blood glucose of the experimental animals is presented in Tables 1 – 5. Five weeks after diabetes induction and treatment with crude fraction, aqueous fraction and methanol fraction led to a significant decrease ($p < 0.05$) in glucose level compared to diabetic control (Tables 1, 2 and 3) respectively. Treatment with chloroform and N-hexane fractions led to no significant decrease at ($P < 0.05$) compared with diabetic control (Table 4 and 5) respectively.

Table 1: Effect of oral administration of crude methanol extract of *Ipomoea asarifolia* leaves on fasting glucose of alloxan-induced diabetic rats for 5 weeks

Groups \ Periods	17mg/Kg	35mg/Kg	70mg/Kg	Control	Diabetic Control
48hr	24.22 ± 0.06	15.89 ± 0.29	27.33 ± 0.06	4.49 ± 0.02	27.09 ± 0.03
Week 1	22.72 ± 0.10*	14.43 ± 0.56*	17.41 ± 0.17*	4.52 ± 0.06	28.39 ± 0.63
Week 2	16.19 ± 0.06*	7.80 ± 0.03*	7.20 ± 0.08*	4.51 ± 0.06	30.15 ± 0.03
Week 3	11.11 ± 0.11*	7.22 ± 0.02*	6.63 ± 0.06*	4.41 ± 0.06	29.56 ± 1.35
Week 4	10.02 ± 0.03*	7.96 ± 0.03*	6.91 ± 0.03*	4.39 ± 0.06	30.41 ± 0.93
Week 5	9.91 ± 0.03*	8.11 ± 0.15*	6.80 ± 0.03*	4.52 ± 0.06	30.74 ± 0.96

Values are presented as mean ± standard deviation. Values with * are significantly differently ($p < 0.05$) compared to diabetic and normal control.

Table 2: Effect of five weeks administration of aqueous extract of *Ipomoea asarifolia* leaves on fasting glucose of alloxan-induced diabetic rats

Subgroups	17mg/kg	35mg/kg	70mg/kg	Control	Diabetic Control
Periods					
48hr	16.87 ±0.45	19.48 ±0.06	22.00 ±0.24	4.49±0.02	27.09 ±0.03
Week 1	29.48 ±0.06 ^{*a}	11.22 ±0.4 ^{*b}	17.48 ±0.08 ^{*c}	4.52 ±0.06	28.39 ±0.63
Week 2	17.22 ±0.11 ^{*a}	8.81 ±0.08 ^{*b}	7.24 ±0.08 ^{*c}	4.51 ±0.06	30.15 ±0.03
Week 3	14.01 ±0.11 ^{*a}	8.31 ±0.28 ^{*b}	6.91 ±0.22 ^{*c}	4.41 ±0.06	29.56 ±1.35
Week 4	11.81 ±0.61 ^{*a}	7.83 ±0.06 ^{*b}	6.94 ±0.15 ^{*c}	4.39 ±0.06	30.41 ±0.93
Week 5	11.81 ±0.61 ^{*a}	7.96 ±0.16 ^{*b}	7.06 ±0.06 ^{*c}	4.52 ±0.06	30.74 ±0.96

Values are mean ± standard deviation. Values with * are significantly different (p<0.05) compared to diabetic and normal control, while values bearing alphabet are significant at p<0.05 compared to the week of no treatment.

Table 3: Effect of 5 Weeks Administration of Methanol Fraction Extract of *Ipomoea asarifolia* leaves on a glucose of alloxan-induced diabetic rats

Subgroups	R1 17mg/kg	R2 35mg/kg	R3 70mg/kg	Control	Diabetic Control
Periods					
48hr	12.65 ±0.08	19.48 ±0.06	20.12 ±0.24	4.49±0.02	27.09 ±0.03
Week 1	11.35 ±0.14 [*]	17.15 ±0.06 [*]	15.02 ±0.03 [*]	4.52 ±0.06	28.39 ±0.63
Week 2	6.67 ±0.06 [*]	6.01 ±0.11 [*]	5.78 ±0.25 [*]	4.51 ±0.06	30.15 ±0.03
Week 3	6.43 ±0.12 [*]	5.41 ±0.37 [*]	4.57 ±0.33 [*]	4.41 ±0.06	29.56 ±1.35
Week 4	5.72 ±0.15 [*]	5.46 ±0.08 [*]	4.02 ±0.13 [*]	4.39 ±0.06	30.41 ±0.93
Week 5	5.26 ±0.22 [*]	4.93 ±0.28 [*]	4.26 ±0.21 [*]	4.52 ±0.06	30.74 ±0.96

Values are mean ± standard deviation. Values with * are significantly different (p<0.05) when compared to diabetic control.

Table 4: Effect of five 5 Weeks administration of Chloroform Extract of *Ipomoea asarifolia* leaves on fasting glucose of alloxan-induced diabetic rats

Subgroups	17mg/kg	35mg/kg	70mg/kg	Control	Diabetic Control
Periods					
48hr	24.11 ±0.06	18.63 ±0.26	11.11 ±0.06	4.49±0.02	27.09 ±0.03
Week 1	30.33 ±0.68 [*]	16.37 ±0.23 [*]	15.28 ±0.25 [*]	4.52 ±0.06	28.39 ±0.63
Week 2	26.65 ±0.03 [*]	14.28 ±0.11 [*]	11.55 ±0.06 [*]	4.51 ±0.06	30.15 ±0.03
Week 3	26.41 ±0.28 [*]	13.48 ±0.48 [*]	11.17 ±1.69 [*]	4.41 ±0.06	29.56 ±1.35
Week 4	17.54 ±1.14 [*]	14.56 ±0.51 [*]	13.83 ±0.87 [*]	4.39 ±0.06	30.41 ±0.93
Week 5	26.13 ±0.03 [*]	21.70 ±0.03 [*]	19.81 ±0.06 [*]	4.52 ±0.06	30.74 ±0.96

Values are mean ± standard deviation. Values with * are not significant at p<0.05 when compared to diabetic control and weeks of no treatment.

Table 5: Effect of five weeks administration Of N-hexane extract of *Ipomoea asarifolia* leaves on fasting glucose alloxan-induced diabetic rats

Subgroups Periods	17mg/kg	35mg/kg	70mg/kg	Control	Diabetic Control
48Hr	22.26 ±0.06	20.96 ±0.13	15.04 ±0.03	4.49±0.02	27.09 ±0.03
Week 1	30.48 ±1.09*	22.17 ±0.06*	13.8 ±0.95*	4.52 ±0.06	28.39 ±0.63
Week 2	28.33 ±0.06*	22.11 ±0.06*	19.44 ±0.06*	4.51 ±0.06	30.15 ±0.03
Week 3	31.15 ±0.06*	20.78 ±0.22*	19.72 ±0.06*	4.41 ±0.06	29.56 ±1.35
Week 4	19.28 ±0.17*	18.67 ±1.84*	17.5 ±1.69*	4.39 ±0.06	30.41 ±0.93
Week 5	29.63 ±0.06*	21.54 ±0.13*	19.81 ±0.13*	4.52 ±0.06	30.74 ±0.96

Values are mean ± standard deviation. Values with * are not significant at $p < 0.05$ when compared to diabetic control and weeks of no treatment.

Discussion

Acute toxicity study reveals the safety of the plant at a dose below 1949mg/kg as none of the animals showed any sign of toxicity at lower dose in both first and second phases of the toxicity study. The plant was found to be slightly toxic at doses of 500-5000mg/kg orally administered. Therefore the plant may be considered slightly toxic according to classification of toxic substances by Bhardwaj and Gupta. [18] The finding of this research work indicated that the plant should be used with caution.

In this study, various fractions of *I. asarifolia* have been analysed for hypoglycaemic effect. The n-hexane and chloroform fractions (hydrophobic fractions) did not appear to have any hypoglycaemic activities as presented in Tables 6 and 7 respectively. The methanol fraction, however, showed profound effect in lowering blood sugar (Table 2). The blood glucose-lowering effect of the methanol extract may be associated with the polyphenols contained in the fraction as it was established that major polyphenols such as flavonoids and tannins are extracted into methanol. [19] Growing evidence from animal studies supports the antidiabetic properties of dietary polyphenols which could be one dietary therapy for the prevention and management of type 2 diabetes. [20] Different mechanisms of action of antidiabetic plants have been proposed such as potentiation of insulin effect either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin, [21] inhibition of hepatic glucose production, [22] inhibition of intestinal glucose absorption [23] or correction of insulin resistance. Diabetes mellitus is a disorder characterised by hyperglycemia and clear evidence

has shown that diabetes and its complication arise due to oxidative stress. [24] The selective destruction of β -cells of the pancreas by alloxan is mediated via generation of reactive oxygen species through its reduction of glutathione to dialuric acid, in which redox recycling process generated reactive oxygen species that culminate in cell damage. [25]

Glucokinase (hexokinase IV) has a major role in the control of glucose homeostasis because it is the predominant hexokinase expressed in the liver [26]. It has very high control strength on hepatic glucose disposal and is the glucose sensor for insulin secretion in pancreatic β -cells. Flavonoids are known to inhibit glucose-6-phosphatase activity in the liver thereby suppressing gluconeogenesis and glycogenolysis and consequently reduce hyperglycaemia. [27] In the present study, flavonoids in the extract may have a stimulating effect on insulin secretion from the remains of β -cells not completely hitherto destroyed by alloxan; however maintenance of glucose tolerance in animals fed with crude methanol and methanol extracts after withdrawal of treatment suggests the possibility of their potential to regenerate β -cells. Therefore the mechanism of anti-diabetic activity of the plant may not be restricted to inhibition of glucose-6-phosphatase activity, but may also be associated with pancreatic cell regeneration.

Conclusion

The findings of this research indicate the presence of hypoglycaemic component in *I. asarifolia* extracted in methanol. The glucose levels in the group treated with methanol fraction did not resort to gradual

hyperglycaemia after the withdrawal of treatment as noticed in groups administered with other solvent extracts.

It is likely the methanol extract in addition to having hypoglycaemic effect may have as well the potential of regeneration of β cells destroyed by alloxan.

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Hepatocurative Effect of Oral Administration of Aqueous Leaf Extract of *Senna occidentalis* in Rats

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Abstract

Awareness on the utilisation of medicinal plants to curtail diseases is on the increase. This study evaluated the phytochemical contents and effect of aqueous leaf extract of *Senna occidentalis* on liver function indices in CCl₄ induced hepatotoxic rats. A total of 32 male albino rats were used for this study. Two rats were used to confirm CCl₄ induced liver damage, while the remaining were divided into six groups of five rats each. Group I, animals were used as normal control while those in groups II-VI were induced with hepatotoxicity. Group II were hepatotoxic control and did not receive the plant extract while groups III, IV and V were orally administered with aqueous extract of *S. occidentalis* at doses of 500mg/kg, 250mg/kg and 166mg/kg for four weeks respectively. Group VI were orally administered with livolin at a dose of 100mg/kg for four weeks. At the end of the fourth week, the animals were sacrificed and their serum activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) as well as serum levels of total bilirubin (TB), direct bilirubin (DB), total protein (TP) and albumin (ALB) were determined. Results of phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides, resins, and flavonoids. The results showed significant ($p < 0.05$) increase in mean serum activities of AST, ALP, and ALT, serum total bilirubin and direct bilirubin levels, and decrease in mean serum TP and albumin levels in Group II when compared with Group I. Results indicated that oral administration of aqueous leaf extract of *S. occidentalis* at the doses of 500mg/kg, 250mg/kg and 166mg/kg for four weeks showed a significant ($p < 0.05$) decrease in the serum AST, ALP, ALT, total bilirubin, direct bilirubin, and significant increase ($p < 0.05$) in TP and albumin when compared with group II. Thus, it could be inferred that aqueous leaf extract of *S. occidentalis* could have hepatocurative effect against CCl₄ toxicity.

Keywords: Liver function, *Senna occidentalis*, Oral administration, Hepatocurative, Rats.

Introduction The use of plant extracts in traditional medicine is a worldwide practice. Medicinal plants form the basis of primary healthcare for the majority of the people living in the rural and remote areas of Nigeria and other third world countries. According to the World Health Organisation (WHO), about 80% of the world inhabitants rely essentially on herbal medicine for their primary healthcare needs.^[1]

Senna occidentalis is one of the plants employed in ethnomedicine in many parts of the world. It is commonly called “Rai-Rai” in Hausa ^[2] and it has a rich history in natural medicine. The parts of the plant used include roots, leaves, and seeds.^[3] In Nigeria, the leaves of *S. occidentalis* are employed in treating various skin infections. The leaf decoction is used for treating typhoid, fever, constipation and also as a diuretic. The seeds of *S.*

occidentalis are also used for treatment of diseases like hypertension, stomach upset, etc.^[4] Livolin is a hepatoprotective drug that is being used in hospitals in the treatment and management of liver diseases. Therefore, this study was aimed at assessing hepatocurative ability of the leaf of *S. occidentalis*.

Materials and Methods

Collection and Preparation of Plant Material *Senna occidentalis* leaves were collected at the old campus of Bayero University, Kano, Nigeria and identified at Plant Biology Department, Bayero University with herbarium number BUK/HAN/073. The leaves were then dried under shade and pulverized into a powder; 200g of the powder was weighed and soaked in 400cm³ of distilled water for 24 hours. The mixture was filtered using Whatman filter paper no. 1 and the filtrate dried. The dried extract was reconstituted for further use.

The volume to be administered to animals was calculated according to the method of Alhassan *et al.* (2015).^[5]

$$\text{Vol. (cm}^3\text{)} = \frac{\text{weight of the rat (kg)} \times \text{dose (mg/kg)}}{\text{Concentration of the extract (mg/cm}^3\text{)}}$$

Experimental Animals

Thirty-two (32) male albino rats weighing between 80g to 120g were housed in cages in a well-ventilated room at room temperature under standard condition and had free access to water and food throughout the period of the experiment. They were fed with standard vital feed diet (Growers pelletized; Grand Cereals Ltd, Nigeria). The experiments were performed according to the principles of laboratory animal care.

CCl₄ induced hepatotoxicity

Hepatotoxicity was induced in rats by administration of 150mg/kg bodyweight of CCl₄ via intraperitoneal injection.

Experimental Design

The experimental rats were given an intraperitoneal injection of CCl₄ (150mg/kg) to induce liver damage; five of the rats were normal control (Group I) and were not administered CCl₄. Two days after CCl₄ administration, two rats were sacrificed to confirm the induction,^[6] while the remaining 25 rats were divided into five equal groups (of five rats each). Group II were administered with CCl₄ and no extract was given (test control) Group III were orally administered with 500mg/kg *S. occidentalis* leaf extract daily for four weeks after CCl₄ induction. Group IV were orally administered with 250mg/kg *S. occidentalis* leaf daily for four weeks after CCl₄ induction. Group V were orally administered with 166mg/kg *S. occidentalis* leaf extract daily for four weeks after CCl₄ induction. Group VI were orally administered with 100mg/kg livoline (standard drug) daily for four weeks after CCl₄ induction.

At the fourth week, all the rats were sacrificed (24 hours after last treatment). Blood samples were collected for analysis of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), total protein (TP) and albumin (ALB).

Qualitative Determination of Phytochemicals^[7]

Test for Alkaloids

The aqueous leaf extract of *S. occidentalis* was rendered slightly acidic. The acetic acid extract was divided into 4 portions, one served as a blank (control). To one portion, few drops of Mayer's reagent (potassiummercuric iodide solution) were added, to the other few drops of Dragendorff's reagent (potassium bismuth iodide) and to the third few drops of Wagner's reagent. A precipitate/turbidity observed in all the tests as compared with the control indicates the presence of alkaloids.

Test for Flavonoids

To 1% hydrochloric acid, extract of *S. occidentalis* (2ml), few drops of 20% NaOH were added. A canary yellow colour indicates flavonoids presence.

Test for Cardiac Glycosides (Baljet's test)

Baljet's reagent was prepared by freshly mixing equal volumes of 1% picric acid in 95% alcohol and 10% NaOH in water. Few drops of the reagent were added to *S. occidentalis* extract (2ml), which gave a light orange to a red colour indicating the presence of cardinolides.

Test for Saponins (Froth test)

S. occidentalis extract (2ml) was shaken vigorously; a persistent froth lasting for 3 minutes indicate presence of saponins.

Test for Tannins

Few drops of 5% ferric chloride (FeCl₃) were added to *S. occidentalis* extract (2ml); a blue-green colour indicates the presence of tannins.

Test for Resins

Extract of *S. occidentalis* (2ml) was dissolved in acetic anhydride (10ml). Concentrated sulphuric acid (1 drop) was added. The presence of purple colour, which rapidly changes to violet, indicates absence of resins.

Analysis of Liver Function

Activities of serum transferases (ALT and AST) were estimated according to Reitman and Frankel (1957).^[8] Serum alkaline phosphatase activity was measured as reported by Rec (1972).^[9] Determinations of serum bilirubin^[10], total protein^[10] and albumin^[11] were also carried out.

Histopathology

Histological examination of the liver was done using a binocular microscope at x100 magnification.

Statistical Analysis

Results were expressed as mean $\pm$ standard deviation and analysed using one-way analysis of variance (ANOVA) in which $p < 0.05$ differences in mean were considered significant.

Results

Phytochemical Screening

Results of qualitative phytochemical screening of aqueous leaf extract of *S. occidentalis* indicated the presence of tannins, flavonoids, saponins, cardiac glycosides, and resins.

Biomarker Liver Enzymes

The results of the effect of aqueous leaf extract of *S. occidentalis* on liver enzymes of rats administered orally with 500mg/kg, 250mg/kg and 166mg/kg aqueous *S. occidentalis* leaf extract after intraperitoneal injection of 150mg/kg CCl_4 are presented in Figure 1. There was a significant increase in the serum AST, ALT and ALP level ($p < 0.05$) in CCl_4 induced control group (group II) compared to the normal control (group I). A significant decrease ($p < 0.05$) in ALT, and ALP was observed in groups treated with 500mg/Kg, 250mg/kg, 166mg/kg extract compared with the group treated with a standard drug (livolin). There was also significant decrease ($p < 0.05$) in AST in the group treated with 500mg/kg, 250mg/kg of the plant extract in a dose-dependent pattern when compared with the group treated with the standard drug.

Non-enzymatic marker

Results of the effect of aqueous leaf extract of *S. occidentalis* on liver TB, DB and IB, TP and albumin of rats administered orally with 500mg/kg, 250mg/kg and 166mg/kg aqueous leaf extract of *S. occidentalis* after intraperitoneal injection of 150mg/kg CCl_4 , are shown in Figure 2 and Figure 3 respectively. There was a significant increase in the serum TB and DB level ($p < 0.05$) in CCl_4 induced control group (group II) compared to the normal control (group I). A significant decrease ($p < 0.05$) in TB and DB was observed in rats orally administered with 500mg/Kg, 250mg/kg, 166mg/kg of the extract in a dose-dependent pattern when compared with the group treated with standard drug (livolin). This is in contrast to total protein (TP) and albumin (ALB) (Figure 3) in which CCl_4 control group (group II) show significant decrease ($p < 0.05$) and increase in groups treated with 500mg/Kg, 250mg/kg and 166mg/kg of the extract in dose-dependent pattern and group treated with standard drug.

Histopathology

The results obtained for histopathological study shows a photomicrograph of cross-section of the liver of a rat in Group I (normal control) (Figure 4) and liver of a rat induced with damage at a dose of 150mg/kg of CCl_4 (group II test control) (Figure 5). Figures 6, 7, 8 and 9 show liver of rats treated with the varying doses of *S. occidentalis* aqueous extract (500, 250 and 166mg/kg and livolin respectively).

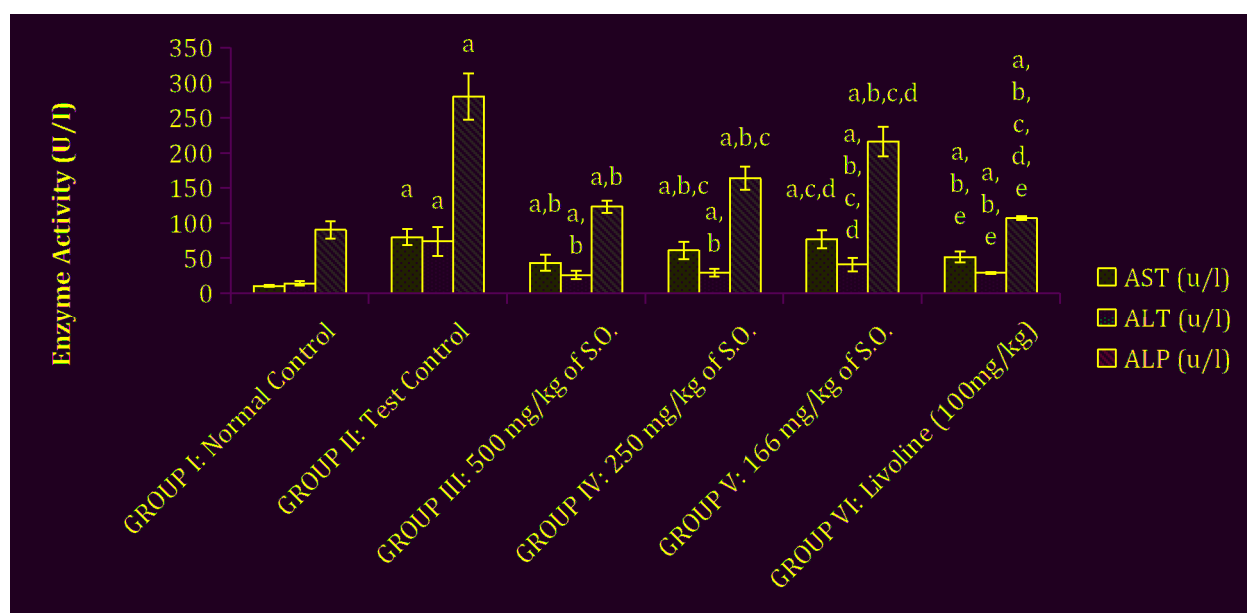


Fig. 1: Effect of aqueous leaf extract of *Senna occidentalis* (S.O.) on the activities of aspartate aminotranferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats administered with CCl₄.

Results are expressed as mean±SD, n=5

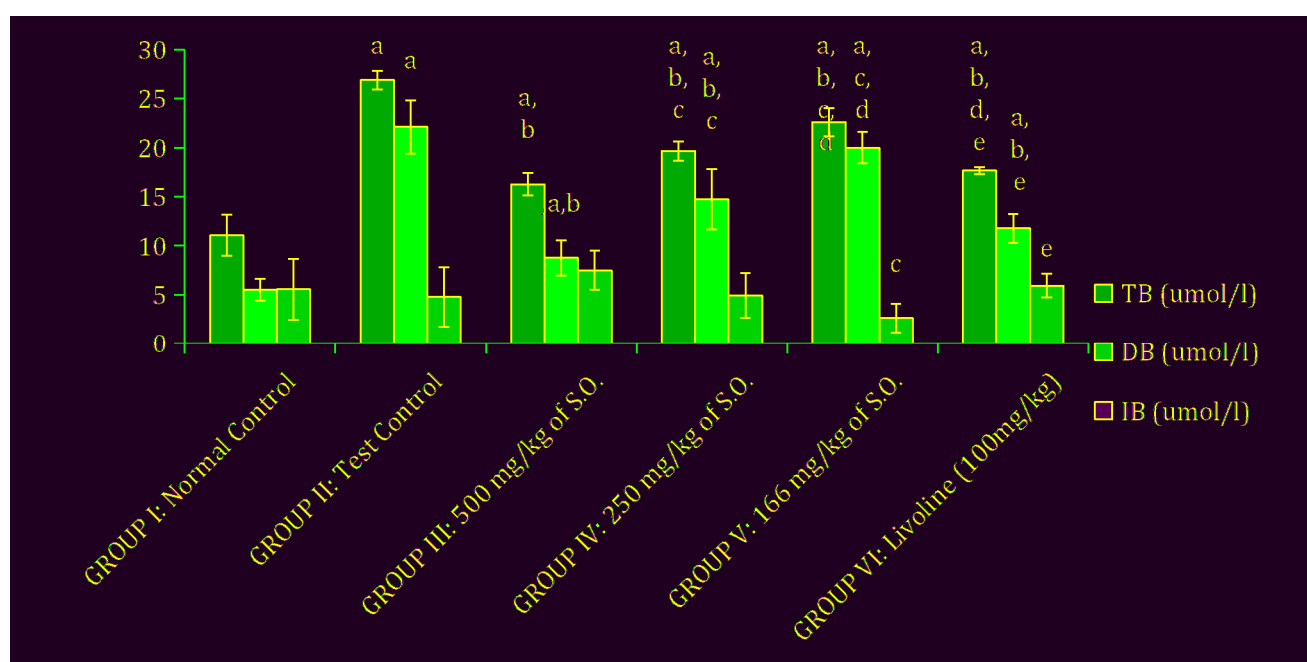


Figure 2: Effect of aqueous leaf extract of *S. occidentalis* on total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB).

Results are expressed as mean±SD, n=5

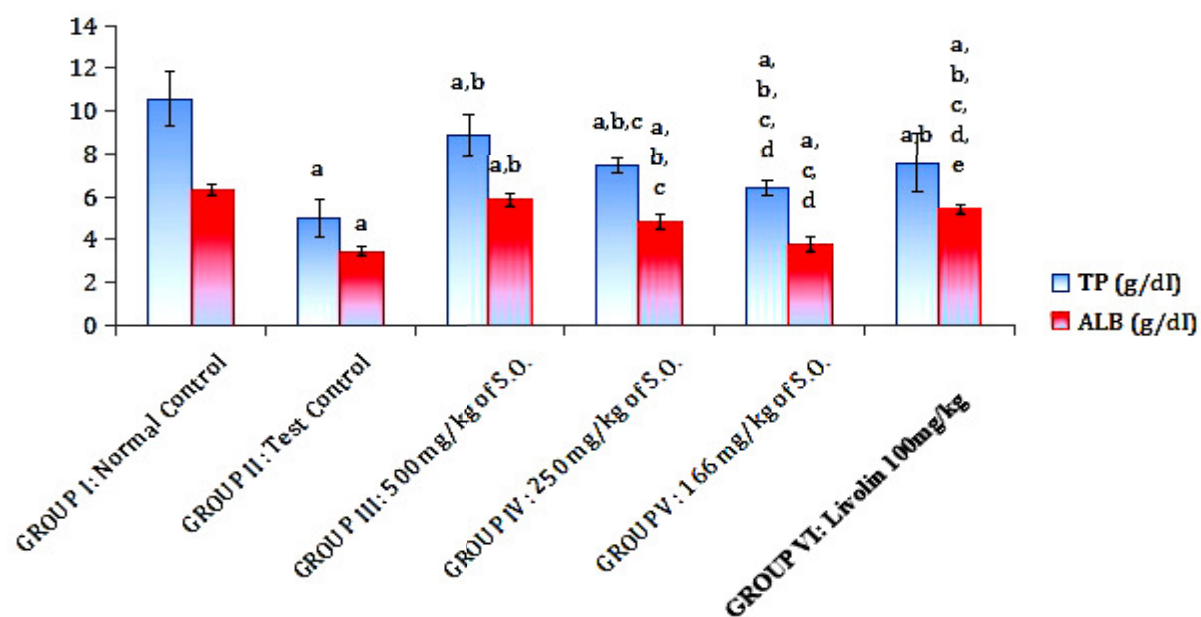


Fig. 3: Effect of aqueous leaf extract of *Senna occidentalis* on total protein (TP) and albumin (ALB).

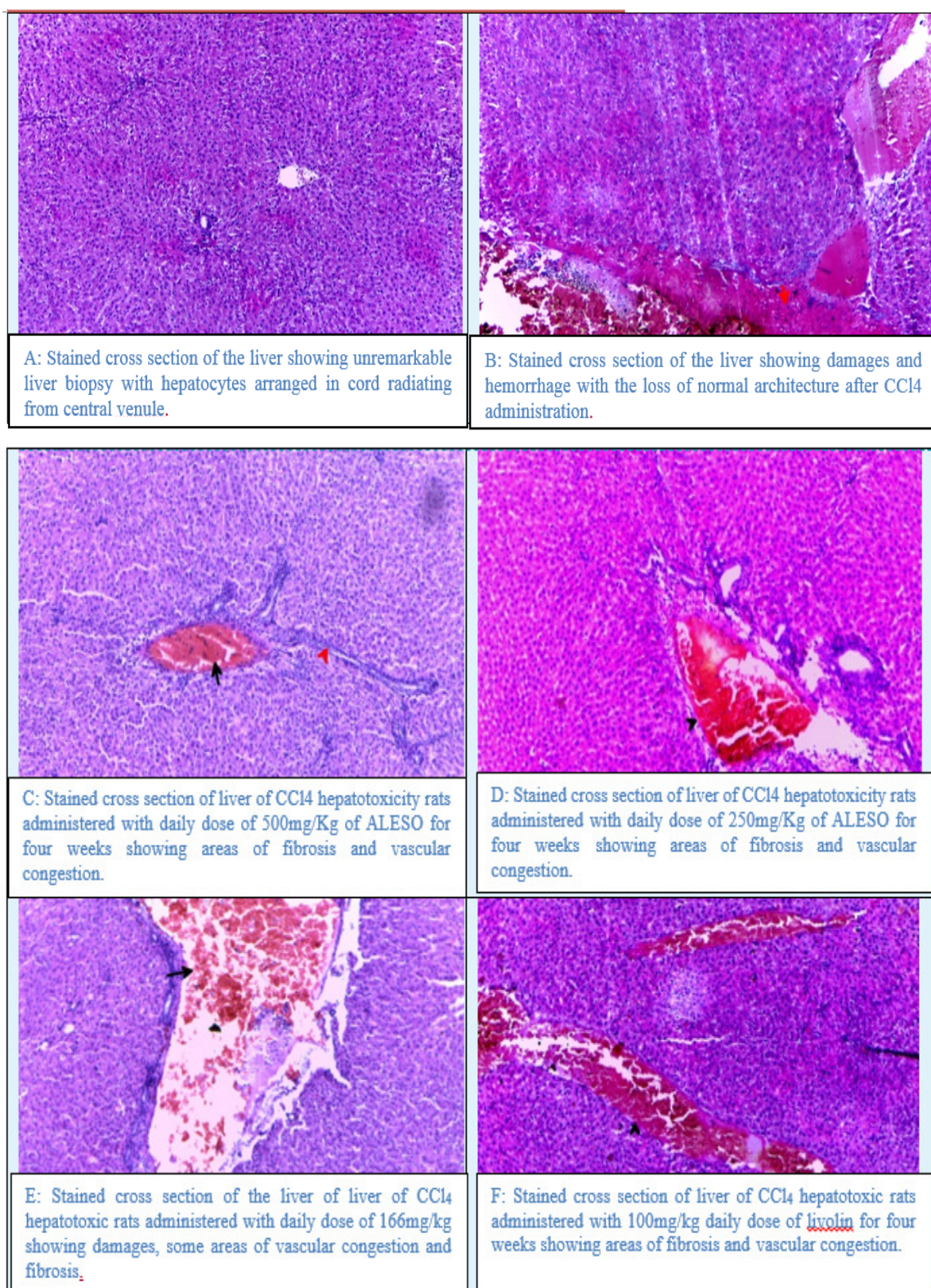


Fig. 4: Histopathological analysis of liver hepatocytes of control and test rats administered with various doses of aqueous leave extract of *Senna occidentalis*. (H and E Stain ($\times 100$))

Discussion

Phytochemical Screening

The result of phytochemical screening of this study shows that *Senna occidentalis* contain tannins, flavonoids, cardiac glycosides, saponins, and resins. This is in accordance with the findings of Grant Grant (1987) [12] that reported the presence of these phytochemicals in *S. occidentalis*. This also agrees with the findings of Toma et al. [17] and Edmund et al. [18] who also reported the presence of the above phytochemicals. Phytochemicals exhibit a diverse physiological and pharmacological effect and have disease preventive properties. The presence of the phytochemical compounds in the plant provides empirical basis for its traditional medicinal uses. [13] Tannins were reported to have hepatocurative potentials due to its anti-oxidant properties. [19] Saponins have an antimicrobial effect and could serve as a precursor of steroidal substances with a wide range of physiological activities.

Hepatocurative Effect

The liver is an important organ in the body, it plays a key role in metabolic processes. Carbon tetrachloride is one of the most widely used chemicals for the induction of hepatotoxicity. In the present study, the result showed a significant increase ($p < 0.05$) in mean serum levels of AST, ALT, and ALP (figure 1), total bilirubin and direct bilirubin (figure 2), with a significant decrease ($p < 0.05$) in mean serum levels of total protein and albumin (figure 3) in test control animals after 150 mg/kg of CCl₄ administration when compared with the normal control animals. Extensive evidence demonstrated that CCl₄ is activated in the liver to highly reactive trichloromethyl radicals which initiate free radical-mediated lipid peroxidation of the cytoplasmic membrane phospholipids causing functional and morphological changes in the cell membrane which leads to accumulation of lipid-derived oxidants causing liver injury. [15]

Elevated levels of AST, ALP, ALT, total bilirubin, direct bilirubin and subsequent reduction of total protein and albumin are indications of the liver dysfunction as a result of the induction with the CCl₄. A decrease in the levels of AST, ALP and ALT in the groups orally administered with the aqueous leaf extract of *S. occidentalis* when compared with

the test control suggest that the extract has hepatocurative effect. This is in line with the reports of Usha et al. [14] and Uzzi and Grillo. [15] The findings of the present work, however, contrast with that of Nuhu and Aliyu [13] who reported an elevation in ALT, AST and ALP activities after treatment with *S. occidentalis* in CCl₄ induced liver damage indicating that the crude extract of the leaves may be slightly toxic as concoction for liver ailments. The induction of the liver damage was confirmed by histopathological analysis of the liver of the intoxicated rats which showed a severely damaged liver. The liver of rats treated with 500mg/kg, 250mg/kg and livoline for four weeks (Figure 4) shows areas of fibrosis and vascular congestion, while for rats orally administered with 166mg/kg aqueous leaf extract of *S. occidentalis* for four weeks shows areas of fibrosis (Figure 8) with a vascular congestion and damaged tissue. The severely damaged and haemorrhagic liver in the test control animals is as a result of the CCl₄ exposure (Figure 5). Exposure to CCl₄ had severe adverse health effects such as causing fulminant, hepatic necrosis and haemorrhage. In this study, CCl₄ induced severe liver damage as represented by significantly elevated serum marker enzymes. This is in agreement with earlier findings that CCl₄ induces liver damage and is also able to alter the antioxidant status of the tissue, which is manifested by the abnormal histopathological changes. [16] The liver at the doses of 500mg/kg and 250mg/kg showed mild damage after four weeks of the extract administration indicating some ameliorative effects of the extract on the organ.

Conclusion

The observed decrease in some serum liver function indices, with a concomitant increase in total protein and albumin in hepatotoxic rats orally administered with aqueous leaf extract of *Senna occidentalis* suggest hepatocurative potential of the plant. This finding could provide some biochemical basis for the use of leaf extract of the plant in the treatment of liver toxicity which could be attributed to the phytochemical content of the plant.

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High Performance Liquid Chromatography (HPLC) - Application in Proteomics and other Fields

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Abstract

Chromatographic techniques have been in existence for more than a decade; they offer a means through which mixtures of different compounds are separated, and all these techniques operate virtually on the same principle of mobile and stationary phases with a partition existing in between. While 'Reverse phase liquid chromatography' is the most commonly employed technique used in various fields, 'Proteomics' employs high-pressure liquid chromatography techniques in separation and characterisation of proteins and peptides. Further advancement in this field will offer benefits to the quality of human life. The purpose of the review is to outline the basic settings of high performance liquid chromatographic techniques, as well as its types and application not only in proteomics but also in other spares of human activities. It has been shown that HPLC (reversed phase) has been one of the most widely used separation techniques, not only in proteomics but also in other fields; and that in proteomics, various proteins and peptides can be separated and analysed.

Keywords: Chromatography, Proteomics, Instrumentation, Application.

Introduction

Chromatography literally means colour writing; it was first described by Tswett Mikhail Semenovitch,^[1] and is referred to as a collective term that comprises several techniques employed in mixture separation into its components, on the basis of their molecular structures and molecular compositions. The whole process (Figure 1) involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture along with it. Components of a sample that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions. This difference in rates causes the separation of various components. Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography); volatile gases (gas chromatography); paper (paper chromatography); and liquids (paper chromatography). This form of technique has been in existence since the mid 19th

century and was first used in plant studies.^[1] Since then various forms of separation techniques have been developed.

The purpose of the review, therefore, is to outline the basic settings of high performance liquid chromatographic technique, as well as its types, and applications, not only in proteomics but also in other spares of human health and other activities. To achieve this, the following tasks were set as objectives:

1. Discuss chromatography in general;
2. Explain what high-performance liquid chromatography is, as well as the components of the system
3. Outline the spares of HPLC application.

The initial technique employed in protein separation involves a two-dimensional gel electrophoresis, which separates proteins based on their molecular masses and iso-electric points. The main disadvantage of it is that, about 50-75% of proteins on the gel could not be detected and as such, the need for developing better detection techniques.

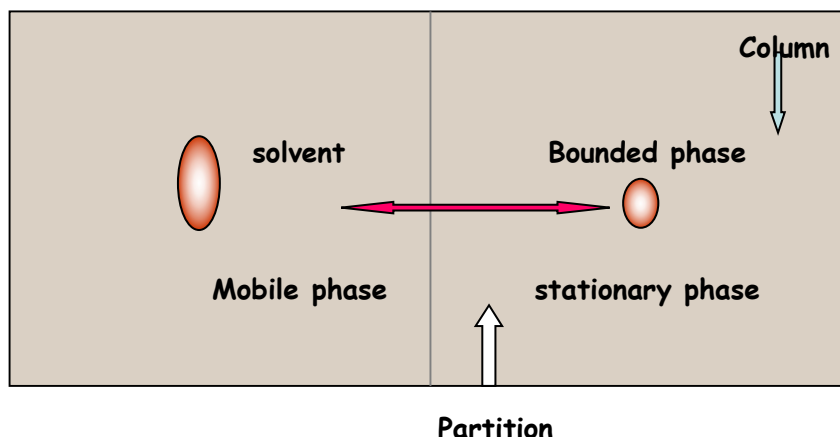


Fig. 1: The main components of a chromatographic system.

Types of Chromatography

Various forms of chromatography, depending upon the phase system (stationary) in the process, have been in existence, and include Thin layer, Gas, Liquid, and High performance liquid chromatographic (HPLC) techniques. More conveniently, chromatographic techniques can be broadly divided into two groups based on the physical property of the mobile phase - liquid and gas types. [2] The basic principle of these two broad groups is mainly the same: A partition exists between the mobile phase the stationary phases. [3]

Normal Phase (NP-HPLC) Technique - The first form of the chromatographic technique was the 'Normal phase liquid chromatography' and was discovered by Tswett Mikhail Semenovitch. [4] By means of this technique, substances or analytes are separated on the basis of their polarity. NP-HPLC uses a polar stationary phase and non-polar mobile phase. The stationary phase is usually silica (an increase in the polarity of the mobile phase leads to a decrease in retention of analytes and conversely, is true for the reverse-phase). [5] Silica in form of gel, is the common bond phase used in the NP-HPLC, [6] thereby forming the column packing. In typical mobile phase, on the other hand, hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these are usually employed. Polar samples are, thus retained on the polar surface of the column packing longer than less polar materials.

Reverse phase HPLC - The stationary phase in this technique is non-polar (hydrophobic) in nature, while the mobile phase, a polar liquid, such as a mixture of water and methanol or acetonitrile (see below). It works on the principle of hydrophobic interactions; hence the more non-polar the material is the longer it will be retained.

Size-exclusion HPLC - The column, in this type of chromatography, is filled with a material having precisely controlled pore sizes, and the particles are separated according to their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and are subsequently eluated.

Ion-exchange HPLC - The stationary phase has a surface, the ions in which are of opposite charges to the ions in a sample; thus the technique is used almost exclusively with ionic or ionizable samples (for example, a sample containing the different fractions of glycosaminoglycans). The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to eluate. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control eluation time.

High-Performance Liquid Chromatography (HPLC)

Amongst the various forms of chromatographic

techniques, the most commonly used is the high-performance liquid chromatography, due to its high selectivity, good separation ability and its diversity in the analyses of high-density mixtures.^[7] In contrast to the normal gravity controlled liquid chromatography, and as the name implies, HPLC is a highly improved form of column liquid chromatography that allows faster delivery and analyses of the mixtures.^[8] Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres; this makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation. The essential requirements for this technique were much earlier proposed by Tswett Mikhail Semenovitch who stated that, "Thus the smallest HETP (highest efficacy) should be obtainable by using very small particles and a high-pressure difference across the columns".

It took almost four decades before the significance of the proposal was realised.

Components of a Conventional HPLC Chromatographic System

There are six basic components of HPLC - (a) the mobile supply system, (b) the pump, (c) the sample injection system, (d) the sample valve, (e) the column, and (f) the detection unit with the help of which processing generated data is carried out (Figure 2). The heart of the system is the column, where separation occurs. Depending on the number of pumps and the composition of the eluent used, HPLC could either be in the form of 'Isocratic' mode or 'Gradient' mode.^[9] While the former uses a single pump to deliver a single eluent with a constant composition under high pressure, in gradient mode, however, more than one pump is employed for the introduction of two or more eluents with a steady change in the composition of the eluent during analysis. Increase efficiency, decrease eluent retention time, and less tailing of the chromatogram are some of the advantages of gradient mode.

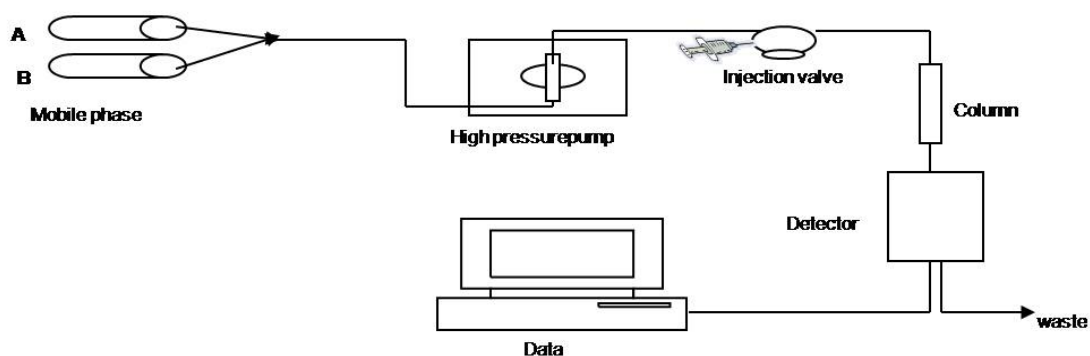


Fig. 2: Schematic illustration of a typical HPLC system.

The Mobile Phase (Solvent Reservoir/ supply system)

Contents of the mobile phase are contained in a glass reservoir and are usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample. As in other forms of chromatography, HPLC has two phases, a mobile phase, and a stationary phase. Two modes of HPLC are nowadays available -the normal phase and reverse phase. Reverse-phase high-performance liquid chromatography is the most commonly

employed separation technique.^[2] The most important feature of this system is the high-pressure pump used for the delivery of the mobile phase, which could be single or multiple, in either isocratic or gradient mode.^[10]

Various solvents have been used in chromatography as preparative agents, which led to a rise in interests in this area.^[11] The common solvents used in the reverse phase include water (H₂O), Methanol (CH₃OH), and acetonitrile (CH₃CN). While Methanol is highly viscous and its miscibility is poor

with non-polar solvents, acetonitrile is less viscous and more compatible with non-polar solvents.^[12] Moreover, retention time is shorter than that of methanol. The solvent composition in the reverse phase is largely determined by thermodynamic equilibrium between the mobile and stationary phases. High-performance liquid chromatography uses a variety of stationary phases,^[5] the common and most widely used in the reverse phase system are usually the silica-based alkyl compounds due to their physical and chemical properties,^[13] which provide a reliable and reproducible result during the technique.^[14] Compounds used include ethyl silyl, octyl silyl, octadecyl silyl and cyanopropyl silyl.

Pumping System

The pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. This is a form of an electro-osmotic pump that is capable of generating a constant flow rate of analytes towards the direction of the column. It operates by generating pressure up to 6000Psi, ensuring a constant and uniform flow of the mixture within the chromatographic system. The flow rate could be controlled within the range of 0.1-10ml/min.^[15]

Sample Injection System

The injector can be a single injection or an automated injection system. An injector for the HPLC system should provide an injection of the liquid sample within the range of 0.1-100 mL of volume, with high reproductivity and under high pressure (up to 4,000 psi). This provides a means through which the sample is introduced into the system. It is usually made up of micro-syringes.^[16]

Columns

These are the main solid support for the bond phases. The property of the column is of utmost importance in an analytic system, from the size of the particles, stability of the particles used, and its ability to reproduce a reliable result.^[17] Column length is usually short and ranges between 25-30cm, with internal diameter of 3-9mm.^[8] They are commonly filled with a stationary phase with a particle size of 3µm-10µm. Ideally, the temperature of the mobile phase and the column should be kept constant during an analysis. Columns with internal diameters of less than 2mm are often referred to as 'microbore columns'. Most HPLC columns are made of silica,

the property of which allows it to interact virtually with all mobile phases, due to its inert nature, enabling it to be used in the separation of the wide variety of chemical compounds.^[18] Another important part of the column is the guard, which is situated anterior to the separating system, so as to filter and remove particles; compounds or ions, which may cause an extraneous peak by interference.^[18] Furthermore, the guard enhances the life span of the column, and in addition, its diameter also influences the efficiency of the column. Columns with smaller diameters have the highest efficiency.^[15] **Detector** – HPLC detector, located at the end of the column detects analytes as they eluate from the chromatographic column. A sample passing through the column is detected by one of the detectors; the commonly used detector in chromatography is the ultraviolet, and it measures the absorbance or emission of the sample.^[19, 20] Ultraviolet detectors detect variable wavelengths, which for most compounds is 250nm. The analytes absorb certain amount of the incident light passing through it from the light source; the absorbance is then detected and recorded as a chromatogram.^[8] The sample and mobile phase are then collected as waste. Other forms of detectors available include chromatography coupled mass spectrometry, electrochemical, nuclear magnetic resonance spectroscopy.^[21, 22]

Data Collection Devices – Signals from a detector may be collected on chart recorders or electronic integrators, which vary in complexity and ability to process, store and re-process chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Peculiarities of High-Performance Liquid Chromatography in Proteomics

Proteome refers to a term derived from proteins that are expressed by the genome. The term was initially used in 1995 to describe the various classes of proteins in cells, tissues, and the organism as a whole.^[23] Since the first description of the proteome, several definitions arose: as the study of proteomes; in 2001 as a branch of science involved with characterising and analysing proteins, their interactions, and modifications in an organism.^[24] Proteome analyses of biological systems became possible through the sequencing of the genome in

humans and other organisms. ^[25] The genome of multicellular organisms has so far been constant and almost similar for all the cells in the same organ or organism and has also been consistent across each species. ^[26] Proteome is dynamic and varies between different cell types of the same organ in an organism, and this is due to the interaction between the genome and environmental factors. ^[27] Moreover, a human cell expresses about 20,000 proteins at any particular time at different dynamic concentrations. ^[28] Other factors that modify proteome expression include growth and developmental conditions, diseases, and drugs. ^[29] Recently, proteomes have been referred to as the protein complement of a genome expressed by a particular cell at a particular time; it also comprises all the isomeric, both co- and post-translational modified forms. ^[26]

The initial technique employed in protein separation involves a two-dimensional gel electrophoresis (2 DE). This separates proteins based on their molecular masses and iso-electric points (see above). ^[30, 31, 32] The main disadvantage of 2 DE is that about 50-75% of proteins on the gel could not be detected and as such, the need for developing a better detection technique (see above). ^[33] Several approaches are now available in proteomics. These include structural proteomics, which entails defining proteins based on their primary, secondary or tertiary structures; functional proteomics, which is used to assess protein function. ^[24] Another form of approach is the profiling proteomics, which analyses the difference in protein expression in healthy versus disease conditions. ^[26]

Instrumentation of HPLC in Proteomics

Reverse-phase-HPLC (RP-HPLC) is the commonly employed separation technique in proteomics; as such, the components are similar to that of conventional HPLC. The efficiency of packing materials plays an important role in separation and resolution of proteins or peptides in proteomics; this is dictated by various factors: size of pore and particles; stationary phase; properties of the surface area and the extent of the surface area involved during the analysis. ^[26] All components of the system, including connecting tubing, flow rate, injection, and volume detection are downscaled in proteomics. ^[34] Flow rates in proteomics are in the nl/min range. The flow system employed to deliver the mobile phase in

proteomics could either be of flow splitting or non-flow splitting. ^[29] While flow splitting employs a high pump in delivering the mobile phase at about 200-330 μ l/min, which is then split within the columns between 100-300nl/min, non-flow splitting system, on the other hand, delivers the mobile phase by means of syringe pumps. ^[29]

As in conventional HPLC, the packing of columns is made up of silica; protein and peptides separation uses silica particles as the stationary phase due to its properties as earlier stated. For an efficient separation in proteomics, the columns used need to be of high resolution, high capacity, and should also be able to perform separation at a faster speed with only little generation of backpressure. ^[35] As such, monolithic columns are more preferred in proteomics due to their robustness. ^[36] Apart from the simplicity of preparation, monolithic column separation media are made up of rigid polymeric rod with an internal porosity. ^[37] There are two types of monolithic columns - the silica group ^[38] and the organic polymer group. ^[39, 40] Organic monolithic columns offer a wide range of advantages, in form of stability of pH, less irreversible adsorption, and ease of preparation and modifications. ^[41] Pre-columns or trap columns are employed by some systems so as to enable injection of large volumes of the highly diluted proteomic samples. ^[42, 43, 44]

Detection Techniques Employed in HPLC of Proteomes

The detection methods used by conventional HPLC are also employed in proteomics. Though proteins and peptides can be detected by the use of online UV absorptions, the wavelength region of peptide bond is strong within the range of 185-220nm, which could not, therefore, detect absorption of the protein and peptides with an aromatic amino acid group, since their absorption is within 275-280nm range. ^[24] Another mode of detection used in proteomics is the fluorescence detection. This also operates within the range of 200-300nm for the aromatic amino acids. ^[24]

Mass spectrometry in proteomics is the ideal detection technique for all the range of proteins and peptides due to its flexibility, sensitivity, and selectivity in the detection of fractionated sample fractions in HPLC. ^[45] It is also employed for protein quantification by means of isotope labelling; more

so, it provides a means of investigating the post-translational modifications of various proteins. [46] Matrix-assisted laser desorption mass spectrometry (MALDI) and electro-spray detectors are nowadays used in characterisation and quantification of proteomes. [30, 32]

Other Applications of High-Performance Liquid Chromatography

The information that can be obtained from HPLC include resolution, identification, and quantification of a substance; it also aids in chemical separation and purification. Other applications include:

Pharmaceutical Applications - Control of drug stability; tablet dissolution of dosage forms' quality control.

Applications in Proteomics - Reverse phase chromatography is the most widely used technique in proteomics since 1976; [47] it provides a means of protein separation. RP-HPLC sequentially offers an opportunity of thorough screening of biological fluid samples such as, and bile or various drugs and their metabolites. [26] Through this, both desired and undesired effects of a drug could be monitored. More so, proteomic approaches could be used for the detection of disease biomarkers as well as for monitoring and evaluating the effectiveness of treatment in a patient. [48] Other recent developments in proteomics involves a technique referred to as 'Surface-Enhanced Laser Desorption /Ionization (SELDI). It involves protein retention on the stationary phase, followed by direct analysis of the time of flight (TOF).

Environmental Applications – Detection of phenolic and other toxic substances in biological samples, as well as bio-monitoring of a variety group of pollutants.

Applications in Forensics – These include: Quantification of drugs in biological samples; identification of steroids in blood, urine, and so on; forensic analyses of textile dyes; determination of narcotics (cocaine and other drugs of abuse in blood, urine, and so on. [26]

Applications in Food Stuffs and Flavours –

Measurement of the quality of soft drinks, water, and so on; sugar analyses in fruit and vegetable juices, as well as analyses of polycyclic substances in the former; analyses of all types of preservatives.

Clinical Applications – Plasma, urine, saliva and antibiotic analyses in blood, as well as those of bilirubin, biliverdin in hepatic disorders; [26] detection of endogenous and foreign neuropeptides in the extracellular fluid of the brain. More so, proteomic approaches could be used for the detection of disease biomarkers as well as for monitoring and evaluating the effectiveness of treatment in a patient. [48]

Other Applications - Other recent developments in proteomics involves a technique referred to as 'Surface-Enhanced Laser Desorption/Ionization (SELDI). It involves protein retention on the stationary phase, followed by a direct analysis of the time of flight (TOF).

Applications of HPLC in the Academics – Certain provisions of the HPLC can be used in teaching special courses in General Biochemistry (structure and function of proteins and methods of research in carbohydrates or in medical biochemistry) laboratory and practical classes relevant in the disciplines of biochemistry, biotechnology, physiology, molecular biology, medical laboratory science, agriculture, and so on.

Conclusion/Recommendations

HPLC has been one of the most widely used separation techniques, not only in proteomics but also in other fields; reversed-phase HPLC is the most commonly employed. In proteomics, various proteins and peptides can be separated and analysed. Clinical proteomics has begun to change the way of drug administration and disease monitoring process. Further development in this field will eventually have a great impact on the health status and quality of human life, in general. As such, advancement in HPLC technology, in form of more sensitive, reliable, and versatile columns, together with excellent detectors will also be of many benefits towards proteomics studies.

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Role of Self-Assembly of Collagen Fibrils in Age-Related Inversion of Connective Tissue Properties in Postnatal Ontogenesis

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Abstract

In most parts of postnatal ontogenesis, structural stability of collagenous formations rises; this is considered as one of the mechanisms of adaptation of connective tissue mechanical properties to an increase in body weight and muscular strength of an organism in the course of its growth and development. The purpose of the study was to investigate the metabolic and functional justifications (changes in the self-assembly of collagen fibrils) to the decrease in the structural stability of matrix sub-molecular complexes of the connective tissue in early postnatal ontogenesis. Investigations were carried out using the dorsal skin of 20 male Wistar rats that were divided into four (4) groups according to their ages. Self-assembly of collagen fibrils was carried out by monitoring their spontaneous formation in saline solution after 1, 3, 10 and 20 days of storage; the presence of fibrils in solution was observed under the microscope. It was found out that, temporary changes in the morphology of fibrils formed from type I collagen of animals of different age groups were significantly different from each other. It was concluded that fibrils formed from the collagen of younger rats were shorter and had smaller diameters than those formed in the collagen of middle-aged and elderly rats; and that, the observed pattern of continued increase in length of fibrils with ageing is the result of age-related characteristic features of processing, and consequently, the nature of cross-linking; during the period up to three (3) months, there is a rapid growth of the newly born organism; because of this, intercellular complexes with high structural stability and rigidity are unfavourable for cellular differentiation and migration, and successful morphogenesis.

Keywords: Collagen, Fibrillogenesis, Ontogenesis, Cross-linkage.

Introduction

The different types of connective tissue are among the most advanced structural composite materials known to consist of macromolecular building blocks. ^[1] A wide range of tissues, each possessing very different properties are successfully synthesized in natural environments with only the same basic macromolecular design. ^[2] Nevertheless, these tissues in many instances show some common features – they are assembled in numerous ways that allow control of the formation of varying hierarchical structures, from nanometre to macro-scale. ^[3] The concept of hierarchical assembly has been recognized and emphasised by more and more scientists over the last decades, as exemplified by the investigations of numerous bio-composite systems. ^[4, 5]

The overall shape and function of the body depend on the basic framework of collagen polymers,

which are usually stabilized by inter-molecular cross-links. ^[4, 5] As a result, collagen fibres are virtually inextensible and provide exceptional tensile strength and resistance to mechanical forces. ^[4, 6] It has been shown that, unlike most other proteins, the turnover of collagen is extremely slow and can, therefore, reflect age-related changes. ^[7, 8, 9, 10, 11] The aim of the present study was to investigate the metabolic and functional justifications (changes in self-assembly of collagen fibrils) to the decrease in the structural stability of matrix sub-molecular complexes of the connective tissue in early postnatal ontogenesis.

Materials And Methods

Grouping of Animals

Investigations were carried out using the dorsal skin of twenty (20) male Wistar rats that were grouped as follows:

Group I – A control group made up of five (5) 1-month old rats (Young animals)

Group II – An experimental group of five (5) 3-month old rats (Young animals)

Group III – An experimental group of five (5) 12-month old rats (Middle-aged animals)

Group IV – An experimental group of five (5) 24-month old rats (Elderly animals)

In carrying out all experiments, clearance on the rules contained in treating animals was obtained, in accordance with International Principles of the European Convention on the protection of vertebrate animals used in experiments and other scientific works. [12] Standards of biomedical ethics, in accordance with the Law of Ukraine on protection of animals from man-handling were as well followed. [13]

Wistar rats were lulled to sleep by intravenous injection of 0.5cm³ of 0.4% solution of sodium thiopental [14] and then decapitated. Dorsal skin samples were cleaned up from hair and dermal fatty layer using acetone and diethyl ether for 24 hours in each case and ground in liquid nitrogen to powder form. Type I collagen was extracted from the powder using a 1M solution of NaCl.

Study of Self-assembly of Collagen Fibrils *in vitro*

Collagen fibrils were obtained by way of their self-assembly. [14] Freshly synthesized type I collagen in 1M solution of NaCl, pH 7.0 was placed in test tubes and stored at 10°C. Spontaneous formation of fibrils was monitored. After 1, 3, 10 and 20 days of storage, the presence of fibrils in solution was observed using a microscope MP-II at 240 magnification, and a digital camera (Canon A410) was used to photograph formed fibrils. Lengths and diameters of fibrils within the field of view were measured on the micrographs obtained; distribution of fibrils having parameters from 0 to a maximum value was plotted. Their relative numbers in a sample in areas of peaks corresponding to individual groups of fibrils were counted.

While calculating areas of integrated peaks of this distribution, contours of respective peaks were arranged into separate components whose shape is described by a linear combination of Gaussian (F_G) and Lorentz (F_L) functions. [15] The uniqueness of the arrangement is achieved by using the number of components observed in the experimentally derived distributions.

Statistical Analyses:

Data were analysed using the software package Origin Pro 7.5 that offered linear and non-linear fittings of self-assembled collagen fibrils; as well as SPSS software, version 20.0 (SPSS) and results expressed as mean values and standard error (± SEM). For normally distributed data, an independent sample t-test was used to compare age-related lengths and diameters of formed fibrils. Differences were considered significant where p<0.05.

Results and Discussion

A three (3) days of storage, individual fibrils and their complexes (fibres) were clearly visible (Fig. 1). While only small isolated fibrils in collagen solution of one-month animals were observed, large and well-formed formations occupying the entire field of view could be seen in collagen solution from 12-months old animals and especially those in 12- and 24-months animals. With an increase in storage time, the number of large fibrillar structures in collagen solution of animals in all four groups increased. [16] However, more intense formation of fibrils from the collagen of older animals compared to 1-month old ones was still observed. [17] This qualitative representation of various types of self-assembly of collagen fibrils of animals of different ages was confirmed by the quantitative data in Figures 1 and 2.

In accordance with the specific primary structure of the polypeptide chains, areas consisting of hydrophobic and polar residues alternate along the surface of the molecule. This distribution of radicals of amino acid residues along the surface of the molecule is the basis of the pronounced ability of type I collagen to self-assembly in the sub-molecular structures – fibrils. [18] Self-assembly towards lateral direction was due to the interaction

of hydrophobic and polar regions, and neighboring molecules became parallel and shifted relative to each other by a quarter of their length.^[19, 20]

Thus, beginning from 3 months of age, and three (3) days of storage in solution, longer fibrils with the maximum distribution of around 1000nm and 1600nm, i.e. corresponding to 1/3 and 1/5 of molecules' lengths, were observed. The number of such fibrils also increased regularly in the collagen solutions from animals in Groups II, III and IV. Fibrils with such lengths appeared in collagen solutions from animals in Group I after 10 days of storage. However, in this case their number continuously decreased in collagen solutions from animals in Groups II, III and IV due to the appearance in them of longer fibrils. The maxima of distribution of longest fibrils that appeared after 22 days of incubation of collagen from animals in all study groups were within the range (1800-1900), (1900-2000), (2400-2800), (2400-3200)nm, respectively. These findings agree with those of Pins., et al, 1994 [21] who found out that, at a microscopic level, self-assembled collagen fibers have wet diameters of ~100µm.

Changes in diameters of fibrils formed from the collagen of animals of different ages were qualitatively similar in nature.^[22] Thus, after three (3) days of storage, maxima of distributions in diameter of all 100% fibrils formed from the collagen of animals in Groups I were within the range of 15-25nm. This is approximately 100-150 molecules of type I collagen arranged laterally.

At the same time, after 3 days of storage, diameters of fibrils that were within the same range but formed from the collagen of animals in Groups IV were already 82.7%. This decrease took place due to the appearance of thicker fibrils with a maximum of distribution 35-40nm in diameter, i.e. those containing up to 300, united side-by-side, molecules.^[16] Similarly, distribution of diameters of fibrils formed from the collagen of animals of different groups after 10 and 22 days of storage

also changed. However, an absolute increase in the diameter of fibrils was less than the one in their lengths. Thus, the maximum average length of fibrils formed from the collagen of animals in Groups IV was four times higher than their average length formed from the collagen from animals in Group I (3200nm and 800nm, respectively). At the same time, a similar relationship for diameters of the fibrils was only 1.7 (40nm and 25nm). This is in line with the findings of Andrzej., et al, 1994 and In-Sik Lee, 2018, who concluded that, the fibrils formed with extracted collagen under physiological conditions tended to narrow and irregular in diameter, and that they lacked the tightly structured appearance of fibrils *in situ*.^[24]

Therefore, the observed pattern of the continuous increase in the length of fibrils formed from the collagen of animals with ageing was the result of age-related characteristic features of processing, and consequently, the nature of cross-linking.^[20, 17] The preferential formation of intermolecular cross-links that bind molecules in fibrils, beginning with the end, in adult and aged animals was the reason behind the increase in diameter of fibrils formed from the collagen of these animals, which was less than the increase in their length. Obtained results could be explained thus, type I collagen molecule is a rigid rod-shaped triple helix of polypeptide chains with a diameter and a length of about 0.15 and 300nm, respectively.^[24, 26, 27]

The biological role or significance of the direction of age-related changes in the self-assembling of fibrils from the skin collagen of young animals lies in the fact that, during this period of development, after a slowdown in growth of the fetus before birth (in early postnatal ontogenesis) during the period up to three (3) months, there is a rapid growth of the newly born organism, and because of this phenomenon, intercellular complexes with high structural stability and rigidity are unfavourable for cellular differentiation and migration, and successful morphogenesis.^[28]

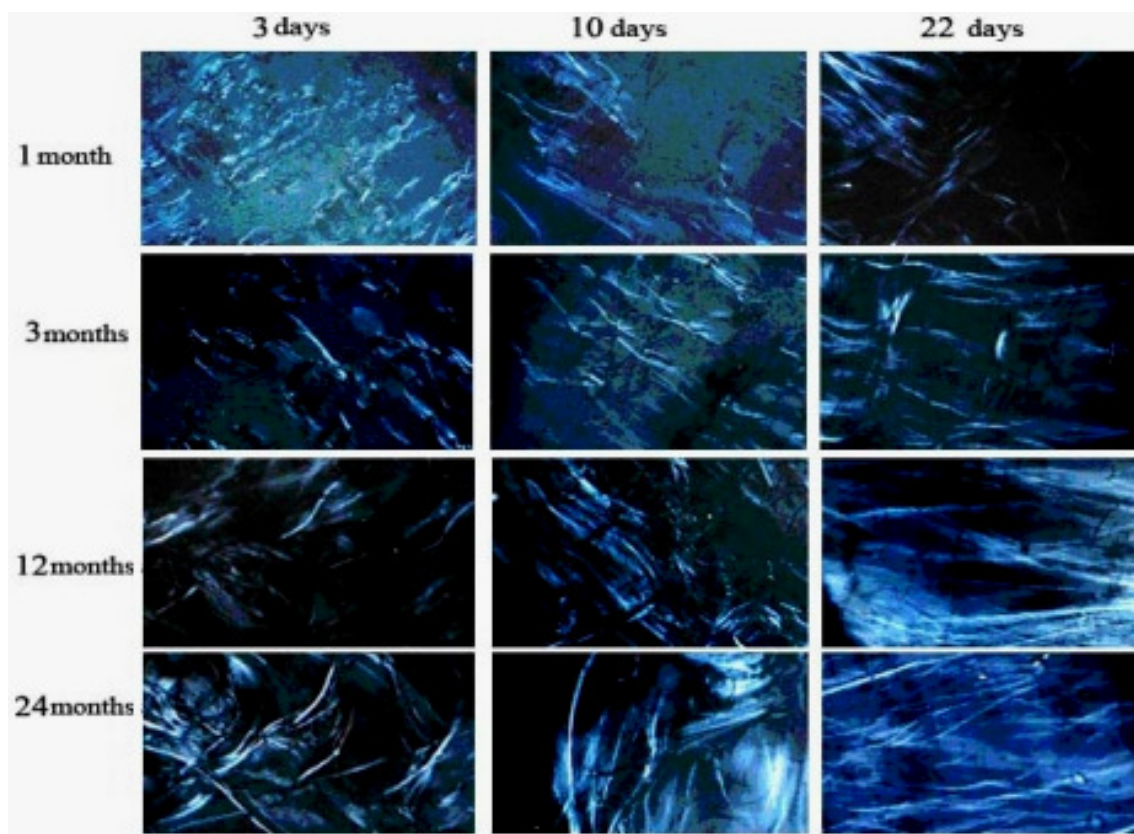


Fig. 1: Micrographs of Fibrils formed from Type I collagen of Rats of different age groups after 3, 10 and 22 days of storage, $\times 240$.

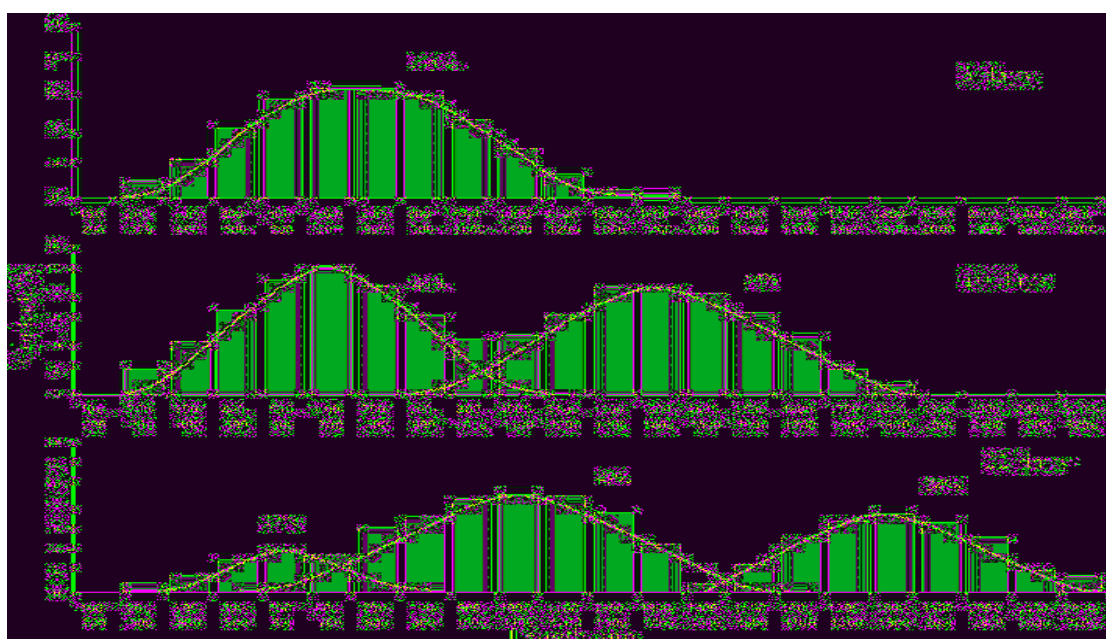


Fig. 2: Temporary changes in the distribution of lengths of fibrils formed from type I collagen of 1-month old animals (Group I).

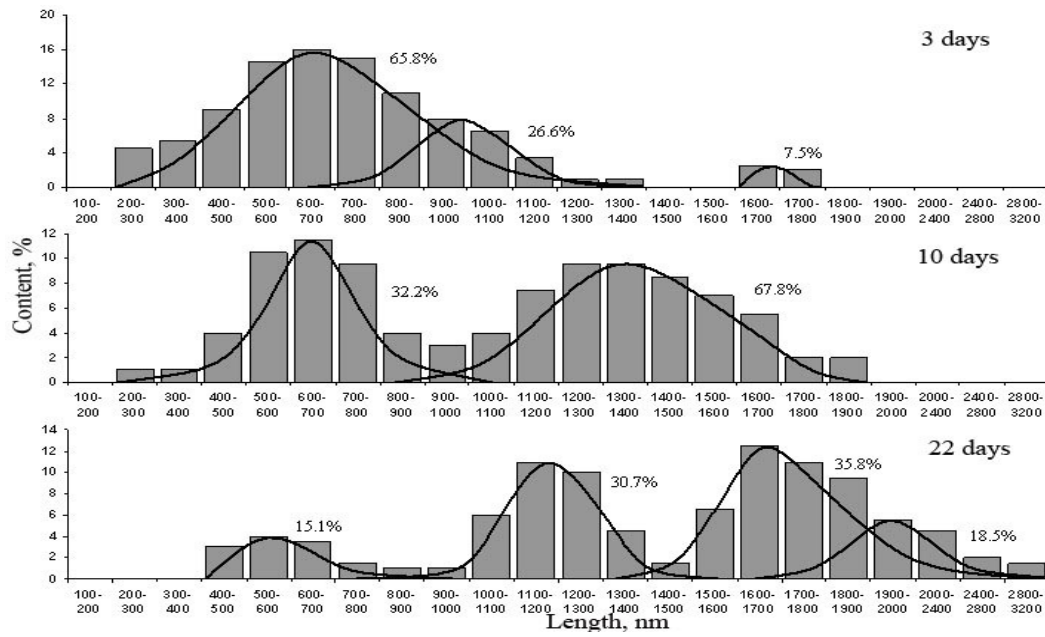


Fig. 3: Temporary changes in the distribution of lengths of fibrils formed from type I collagen of 3-months old animals (Group II).

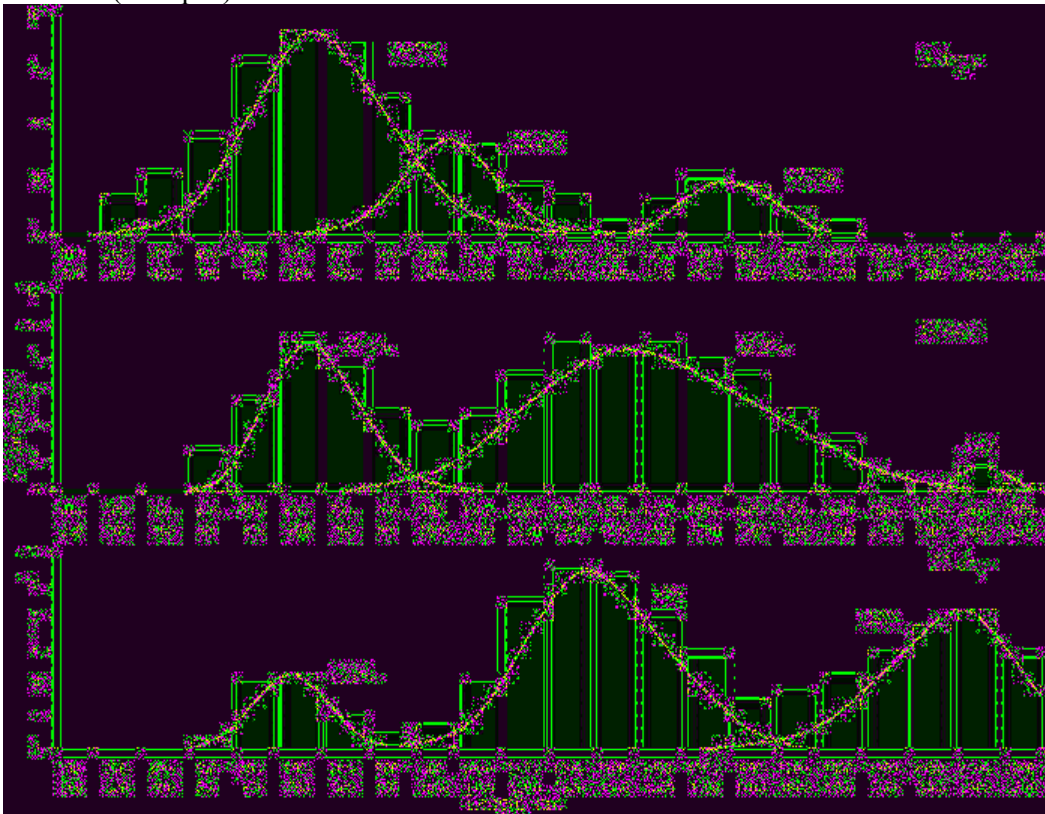


Fig. 4: Temporary changes in the distribution of lengths of fibrils formed from type I collagen of 12-months old animals (Group III).

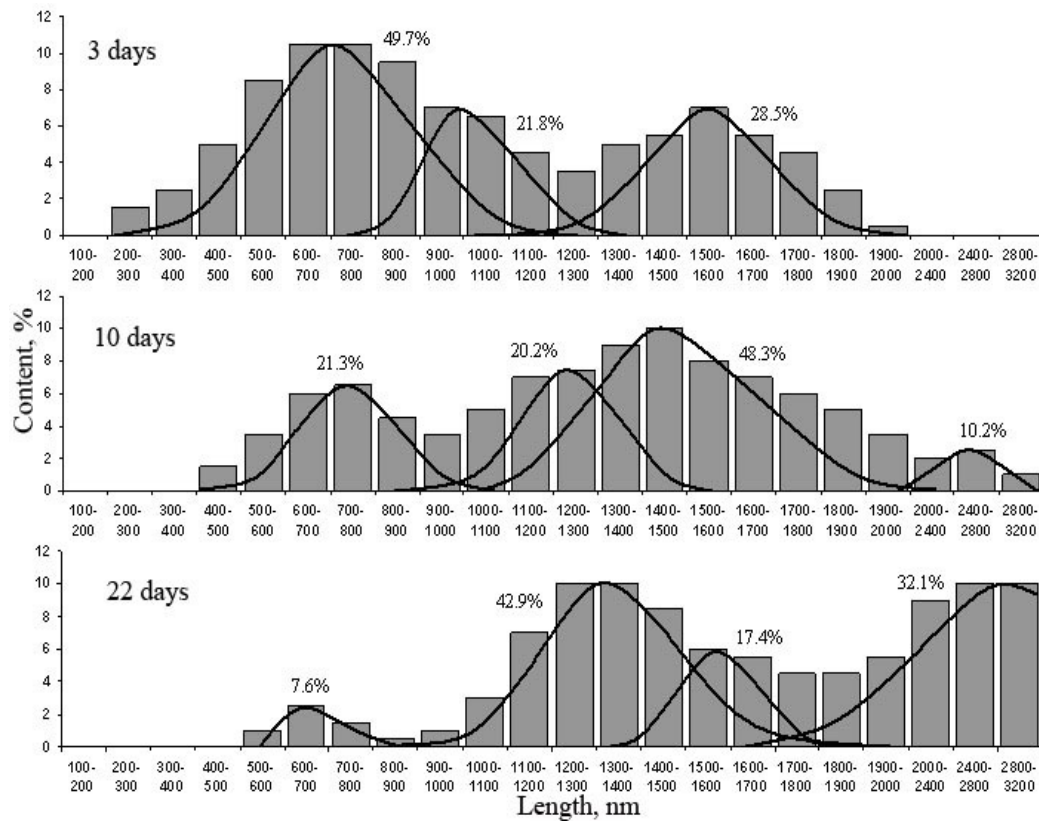


Fig. 5: Temporary changes in the distribution of lengths of Fibrils formed from type I collagen of 24-months old animals (Group IV).

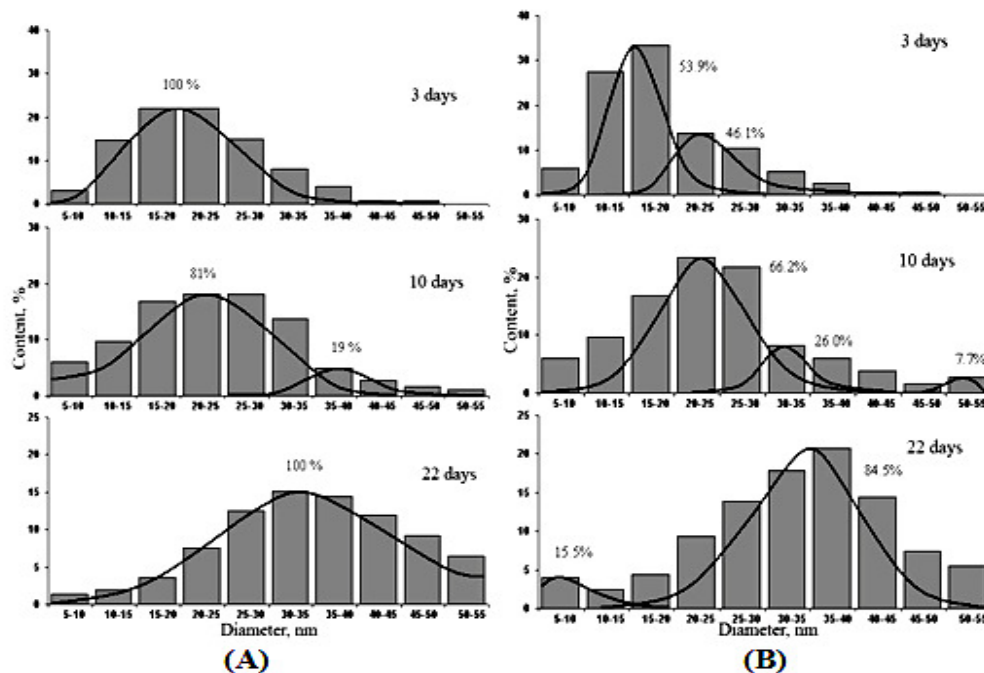


Fig. 6: Temporary changes in the distribution of diameters of Fibrils formed from type I collagen (A) of animals in group I and (B) of animals in group II.

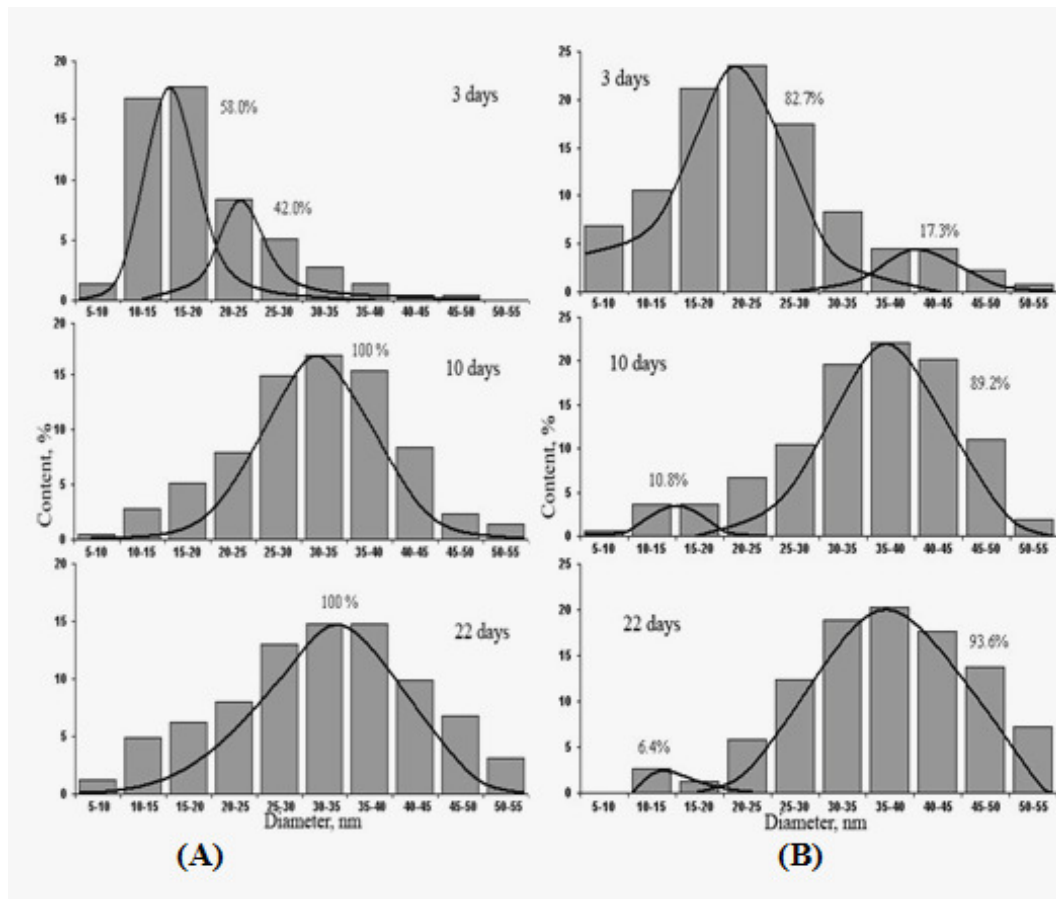


Fig. 7: Temporary changes in the distribution of diameters of Fibrils formed from type I collagen of animals in group III (A) and of animals in group IV (B).

Conclusion

Fibrils formed as a result of in vitro self-assembling from the skin collagen of young animals (Groups II and III; 1- and 3-months old, respectively) were shorter and had smaller diameters than fibrils formed in the skin collagen of middle-aged and elderly animals (Groups III and IV; 12- and 24-months old, respectively). The biological role of this direction of age-related changes in the self-assembling of fibrils lies in the fact that, during this period of development, after a slowdown in growth of the fetus before birth (in early postnatal ontogenesis) during the period up to three (3) months, there is a

rapid growth of the newly born organism, and because of this phenomenon, intercellular complexes with high structural stability and rigidity are unfavourable for cellular differentiation and migration, and successful morphogenesis.

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Serum Sialic Acid Levels in Hormone-based Contraceptive Users Attending Aminu Kano Teaching Hospital, Kano, Nigeria

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Abstract

Hormone-based contraceptive use is on the increase globally, especially in the northern part of Nigeria where it is gaining wide acceptance. However, its use is associated with some side effects such as the increased risk for cardiovascular diseases. Serum sialic acid has been reported to be a cardiovascular risk marker. The aim of this study, therefore, was to evaluate the effect of hormone-based contraceptives on serum sialic acid level as a possible marker for cardiovascular diseases in 162 women attending Aminu Kano Teaching Hospital, Kano, Nigeria. A well-structured questionnaire was administered to each participant and anthropometric data was taken using portable height and weight scale. The mean concentrations of sialic acid were 727.78 ± 73.53 $\mu\text{g/mL}$ and 513.13 ± 63.94 $\mu\text{g/mL}$ for users and controls, respectively. The serum sialic acid showed a significant positive association with hormone-based contraceptives among women ($p < 0.05$). There was also a significant correlation between serum sialic acid levels and duration of use ($p < 0.05$) but no significant correlation was observed based on the type of contraceptive used ($p < 0.05$). This study shows that serum sialic acid levels was increased significantly in users compared with controls. No such significant increase was observed based on the type of contraceptive used although serum sialic acid level appears to increase with duration of use. This data could be used to create awareness among users of hormonal contraceptives about its relationship with increased risk of cardiovascular diseases.

Keywords: Sialic acid, Cardiovascular disease marker, Hormone-based contraceptive, Cardiovascular diseases

Introduction

Hormone-based contraceptives are birth control methods that act on the endocrine system. The majority of all methods are composed of steroid hormones. There are two types of hormonal-based contraceptive formulations: combined methods that contain both an estrogen and a progestin, and progestogen-only methods that contain only progesterone or one of its synthetic analogues (progestin).

Combined methods work by suppressing ovulation and thickening cervical mucus^[1] while progestogen-only methods reduce the frequency of ovulation^[2] most of them rely more heavily on changes in cervical mucus.

Women who take oral contraceptives have an increased risk of cardiovascular diseases compared to non-users.^[3] Birth control pills containing

levonorgestrel and 30mg of estrogen are the safest form of hormonal contraception with regard to the risk of myocardial infarction or ischemic stroke.^[4] Women who use birth control pills are at a slightly increased risk of having a thrombus in the legs or lungs. Studies consistently show that the risk of venous thromboembolism is two to six times higher in oral contraceptive users than in non-users. Other risk factors include obesity and age.^[5] The highest risk of venous thromboembolism is found in pills containing desogestrel and cyproterone.^[6] Combined oral contraceptives can increase the risk of certain types of cardiovascular disease in women with a pre-existing condition or already heightened risk of cardiovascular disease. Metabolic conditions like diabetes, obesity and family history of heart disease are all risk factors that may be exacerbated by the use of certain hormonal contraceptives.^[7]

Sialic acid is a general name for the N- or O-substituted derivatives of neuraminic acid. Neuraminic acid is a monosaccharide with a nine-carbon backbone.^[8] Sialic acids are found in all vertebrates existing in all tissues ^{[9][10]} and in many human body fluids such as breast milk, gastric juice, saliva, serum, urine, and tears.

There were several reports on the role of serum sialic acid in cardiovascular diseases published in the 1970s and 1980s but the real interest in serum sialic acid in cardiovascular disease grew only after the report of Lindberg *et al.* ^[11] which showed that serum sialic acid is a strong predictor of cardiovascular mortality and may also reflect the activity of an atherosclerotic process. Later on, many studies came up showing that serum sialic acids were elevated in cardiovascular diseases. ^[12, 13, 14, 15, 16]

A study by Crook *et al.* ^[17] showed that serum sialic acid significantly correlated with systolic blood pressure, fasting serum cholesterol and triglycerides and body mass index in females. Zahedi *et al.* ^[18] found that serum sialic acid increased post-prandially giving further ideas as to why it is considered to be a cardiovascular risk factor.

The finding that blood pressure correlates positively with serum sialic acid may explain in part why sialic acid predicts cardiovascular mortality.^[11]

Other cardiovascular risk factors are also known to be reflected in serum sialic acid concentration, such as sialic acid-containing acute phase protein fibrinogen.^[19] A possible explanation of these findings is that serum sialic acid reflects the acceleration of the atherosclerotic process by increased blood pressure, which is a known independent risk factor for atherosclerosis.^[20]

From these reports, it seems that irrespective of the mechanism, serum sialic acid seems to be a causal risk factor and could prove useful in screening of population at cardiovascular risk.^[21]

Materials and Methods Study Subjects

A total of 162 subjects attending the Family planning unit of Aminu Kano Teaching Hospital were

randomly selected for the study (mean age of 28.97 ± 7.18) and mean BMI of (25.30 ± 6.58) . Out of the 162 women, 81(50%) were on contraceptive use and 81(50%) served as control groups.

Sample Collection and Preparation

Five-milliliter blood samples were taken from 162 subjects into gel separator tubes. After clotting, blood samples were centrifuged at 3,000 rpm for 10 minutes. Serum was aliquoted into plain tubes and stored at -20°C until use.

Methods

Serum sialic acid was measured using the thiobarbituric acid (TBA) method of Warren (1959). Sialic acid is oxidized to formylpyruvic acid which reacts with thiobarbituric acid to form a pink colored product; the color intensity at 549nm is directly proportional to the sialic acid concentration in the sample. To 125 μl of serum, 1ml of 0.5M HCl was dispensed and incubated at 80°C for one hour followed by addition of 250 μl of periodate reagent and incubated at 37°C for 30 minutes in a water bath. Excess periodate was reduced with 200 μl of sodium arsenite reagent followed by addition of 2ml of thiobarbituric acid. Thereafter, the mixture was incubated in a boiling water bath for 7.5 minutes, allowed to cool followed by addition of 2.5ml of acid butanol reagent. The contents were then centrifuged at 3000rpm for 5minutes to facilitate rapid separation of the two phases. The colour intensity of the butanol layer was measured at 549nm.

A sialic acid calibration curve was used to extrapolate and obtain the sialic acid concentration.

Statistical Analysis

The data analysis was performed using SPSS Statistics software version 20. The mean and standard deviation (SD) was calculated and results expressed as mean $\pm$ SD. The student t-test was used to compare differences between mean values. Correlation between variables was assessed using a paired sample t-test. Statistical significance was set at $p < 0.05$.

Results

There was a significant difference between mean

serum sialic acid of women on hormone-based contraceptives ($727.78 \pm 73.53 \mu\text{g/ml}$) and that of the control group ($513.83 \pm 63.94 \mu\text{g/ml}$; $p < 0.05$) (Table 1). There was no significant difference between the mean value of injectable and that of oral contraceptive users ($738.33 \pm 43.25 \mu\text{g/mL}$ and $720.05 \pm 99.13 \mu\text{g/mL}$ respectively, $p < 0.05$) (Table 2).

As presented in Table 4.3, there was a significant difference in serum sialic acid levels based on the duration of contraceptive use. In users of contraceptives for five years or less serum sialic acid level was $719.14 \pm 68.06 \mu\text{g/mL}$ while in users for more than five years it was $762.88 \pm 86.23 \mu\text{g/mL}$ ($p < 0.05$).

Table 1: Serum sialic acid levels in hormonal contraceptive users and non-users.

Parameter ($\mu\text{g/ml}$)	Users (n=81)	Control (n=81)	p-value
Serum Sialic acid	727.78 ± 73.53	513.83 ± 63.94	0.05

Values are mean $\pm$ SD

Table 2: Serum sialic acid level in injectable and oral contraceptive users.

Values are mean $\pm$ SD

Parameter	Injectable (n=38)	Oral (n=43)	p-value
Sialic acid ($\mu\text{g/ml}$)	738.33 ± 43.25	720.05 ± 99.13	0.05

Table 3: Comparison of serum sialic acid levels on the basis of duration of use of hormonal contraceptive.

Parameters ($\mu\text{g/mL}$)	≤ 5 years (n=65)	> 5 years (n=16)	p-value	r
Sialic acid	719.14 ± 68.06	762.88 ± 86.23	0.05	0.395

Values are mean $\pm$ SD

Discussion

In the present study, serum sialic acid level showed a positive relationship with hormone-based contraceptive use. This is in accordance with previous studies where inflammatory glycoproteins and serum lipids were found to increase with hormonal contraceptive use.^[20,22,23,24] Raised sialic acid levels in the serum could lead to increased sialylation of glycoproteins and glycolipids^[18] and triglycerides.^[17] Hormonal contraceptives contain synthetic estrogenic hormones. Estrogenic hormones increase the serum level of sialic acid-rich inflammatory glycoproteins such as C-reactive protein, haptoglobin, $\alpha 1$ -antitrypsin, and $\alpha 1$ -acid glycoprotein.^[25] This could be attributed to the fact that increased serum total sialic acid leads to elevation of sialic acid-rich C-reactive protein and other sialylated inflammatory glycoproteins in contraceptive users.

Oral contraceptives users were found to have lesser serum sialic acid levels than injectable users. Injectable contraceptives are more effective, have a higher dose of progestin and longer duration of action than oral contraceptives^[26]. Therefore injectable contraceptives have more effect on the degree of sialylation of serum lipids total cholesterol and inflammatory glycoproteins than oral contraceptives.

Previous findings of^[27, 28, and 29] reported high levels of triglycerides, total cholesterol, low-density lipoprotein, very low-density lipoprotein, and high-density lipoprotein cholesterol in injectable contraceptive users. These findings might be related to those of the present work as the observations could be attributed to the effect of injectable contraceptives which may have caused the elevation

of this sialic acid-rich lipids in injectable users. Our findings also revealed a positive association between serum sialic acid level and long-term duration of hormonal contraceptive use. This agrees with the report of Maritta *et al.* [20] showing a positive association between sialic acid level and duration of use. A possible explanation to the observation could be due to the long term effect of progestogen leading to increased level of sialylated low-density lipoproteins and inflammatory glycoproteins.

Conclusion

Serum sialic acid level in hormone-based contraceptive users attending Aminu Kano Teaching Hospital was found to be significantly higher than in

non-users. Serum sialic acid was also found to increase significantly with duration of contraceptive use. Hence hormonal contraceptives have significant effect on level of serum sialic acid in users and also the duration of use, this data could be used to create awareness to the users about its effect on sialic acid level which is a potential cardiovascular disease marker.

Author's Contributions

H. L. Abdullahi conceptualised the study; M. Ahmad and M. M Usman designed the experiments; M. Ahmad collected and process all the blood samples; M. Ahmad conducted the statistical analysis while M. Ahmad and H. L. Abdullahi drafted the manuscript to the present form.

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Study of Cephalic Index Using Computed Tomographs among the Hausa-Fulani Tribe in Kano, Nigeria

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Abstract

The practical application and validity of cephalometric results have made researchers to continually explore newer techniques in the investigation of the craniofacial skeleton. Cephalometry of the Hausa-Fulani tribe has been studied by so many researchers using callipers and lately radiographs. However, cephalometry of Hausa-Fulani tribe in the study area using Computed Tomography (CT) is scanty despite its added advantages. To determine the mean cephalic index of the Hausa-Fulani tribe and to establish if sex differences exist in cephalic index among Hausa-Fulani tribe in Kano. Following ethical approval, a prospective study was conducted at Aminu Kano Teaching Hospital's (AKTH) CT-suit unit. A total of 153 adult head scanograms having satisfied the inclusion criteria were recruited from June - September 2017. Scanograms were used for measuring variables following acquisition by 160 slice Toshiba Prime Aquilion CT-scanner. Cephalic width was measured on the posterior-anterior (PA) scanogram while cephalic length was measured on lateral head scanogram. The cephalic index was determined from variables measured and head shape was classified according to international classification standard of head shape. Male and female Hausa-Fulani in Kano have the dolicocephalic head shape with mean CI of 68.30mm and 68.77mm respectively. No statistically significant difference was established between the sexes ($P>0.05$). Hausa-Fulani tribe has the dolicocephalic head shapes and they do not vary among their sexes. Current findings from this study will be of importance in forensic science and medicine, medical imaging, physiotherapy, and orthopaedic gadgets developments, clinical practice (plastic surgery) and anthropological studies.

Keywords: Cephalic index, Cephalometry, Computed tomography,

Introduction

No two individuals are exactly alike in all their measurable traits, even genetically identical twins (monozygotic) differ in some respects.^[1] The human skeletal dimensions are affected by ecological, biological, geographical, racial, sex and age factors.^[2] Anthropometry is the science of systematic measuring methods that quantitatively measures the dimensions of the human body and skeleton. This science and practice had a long tradition of use in biomedical sciences and its finding increased use in medical sciences especially in the discipline of forensic medicine.^[3] Cephalometry is an important aspect of physical anthropometry in which the

dimensions of head and face are objectively determined.^[4,5] It continues to be a versatile technique in the investigation of the craniofacial skeleton because of its validity and practicality.^[6] It makes use of landmarks on the skull to take specific measurements, which are used to calculate indices.^[2,3,7]

Cephalic Index (CI) is an important parameter for determining and analysing ethnic and sex differences in a population especially individuals whose identity is unknown.^[2] Differences in CI between parents, off-springs, and siblings can give information about

the genetic transmission of the inherited characters.^[8] In forensic medicine, cephalometry is used in age and stature estimation, when only fragment remains are available, in mass disasters and sex determination.^[2,3] It is also largely applied in plastic surgery to repair injuries or defects to the head and face regions.^[1,9]

For several decades, the cephalometric analysis was performed manually using various types of manufactured calipers on dry skulls or living heads. However, since Holly Broadbent in 1931 introduced cephalometric radiography into orthodontics, it received significant attention.^[10,11] Image analysis was performed by tracing radiographic landmarks by measuring linear and angular values using a protractor.^[9,12] This method has been used in investigating the growth and development of cranial and facial skeleton and further assist medically in treatment planning and changes between pre- and post-treatment measurements.^[12] However, despite its widespread adoption, some drawbacks are attributed to the technique.^[12,13]

They include time-consumption, high-risk of error in tracing, landmark identification difficulty, measurement reproducibility error and most importantly superimposition of craniofacial structures.^[12-14] These limitations are grouped into acquisition, identification, and technical measurement errors in medical imaging.^[14] In recent years, diagnosis and treatment planning in medical imaging has been revolutionized by the computerization of image acquisition and processing. With the evolution of digital radiography, digital tracing has slowly replaced the manual tracing methods and its results highly reproducible.^[9,11-14]

Digital radiography has provided several advantages, such as instant image acquisition, reduction of radiation dose, facilitated image enhancement and archiving, elimination of image magnification attributable to radiographic films, facilitated image sharing and simplifies landmark measurements. Image archiving is also a valuable method for overcoming the problem of film deterioration, which has been a major source of information loss in craniofacial biology especially in cases where researchers query results.^[12] These are all features attributable to a CT-scan images including three-

dimensional (3-D) reconstructions. Clinicians treating patients with craniofacial anomalies often use a localization scan, called a CT scanogram, to produce a survey scan of the region of interest.^[15] This solves the issue of superimposition associated with conventional radiographs as it allows focusing of anatomic structures accurately. Hence, scanograms proffers clearer images, more precision, and reliability during the visualizing of landmarks compared with conventional cephalometric radiographs.^[16] Measurements made on CT images, are more accurate, reproducible and have the potential to aid in the craniofacial diagnosis of facial asymmetries, functional shifts, and canted occlusal planes.^[17]

Despite these obvious CT advantages, most works on CI across Nigeria were carried out using different types of calipers or measuring tapes to obtain data from either plain radiographs or dry skulls which are all prone to high measurement error.^[13] Recognition of these factors and other limitations of existing methods justifies the need for this study. The present study aims to determine the CI of the Hausa-Fulani tribe using CT head scanograms, to know the head type, to assess for sex differences and to compare with other populations/tribes.

Materials and Methods

This was a prospective study that was conducted between June - September 2017 in the CT suite of Muhammad Sanusi Radio-Diagnostic Centre, a unit under the Radiology department of Aminu Kano Teaching Hospital (AKTH), Kano. The hospital serves as a referral center to almost all health facilities in Kano and neighbouring states. Kano is situated in the Sahelian geographic region, south of the Sahara. It is the commercial nerve center of Northern Nigeria and is the third-largest city in Nigeria after Lagos and Ibadan.^[18] According to 2006 census figures, it is the most populous state in Nigeria, with about 9,383,682 million people.^[17] Presently, these figures are projected to have raised significantly. The Hausa-Fulani are the dominant tribe in the city and in most parts of Northern Nigeria, Hausa is widely spoken language in Kano.^[18]

Due to the inter-marriages between the inhabitants of the study area, participants selection was based on

those whose either two parents were Hausa and the other Fulani in origin. They were also ruled out for any history of trauma, physical deformities or surgery of the head. Following ethical approval from the research ethics committee of AKTH with reference number: NHREC/21/08/2008/AKTH/EC/2126, 153 normal CT-scanograms comprising of 100(65.3%) males and 53(34.6%) females were recruited using simple random sampling techniques.

Their age ranged from 18-87 years with mean age of 36.8years. The CT-scanner was a 160-slice Toshiba Prime Aquilion with the following features: 512×512 reconstruction matrices, 203×243×107cm gantry size

and 78cm gantry opening. Posterior-anterior (PA) and lateral scanograms were acquired with participants lying supine on the couch; the head was placed in the gantry with the Frankfurt horizontal plane perpendicular to the gantry, gantry tilt at 0°. Scanograms were acquired using the following parameters: 120 kVp, 380 mA, 1s rotation time and 1 mm slice thickness. Participants' confidentiality was guaranteed by the activation of partial image anonymity feature which masked the subject's name, examination number, and clinical diagnosis. Following image acquisition, scanograms were saved in DICOM format and transferred into rewritable compact discs for processing and cephalometric data analysis.^[19]

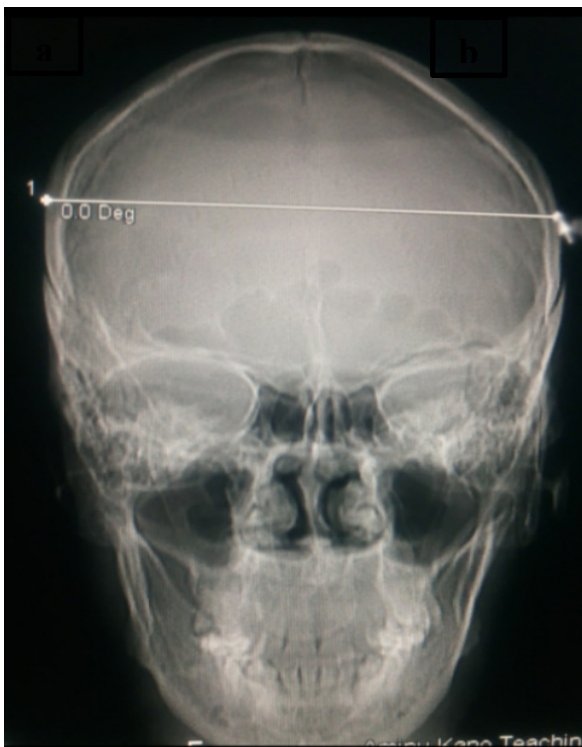


Fig. 1a: Frontal scanogram measuring cephalic width.

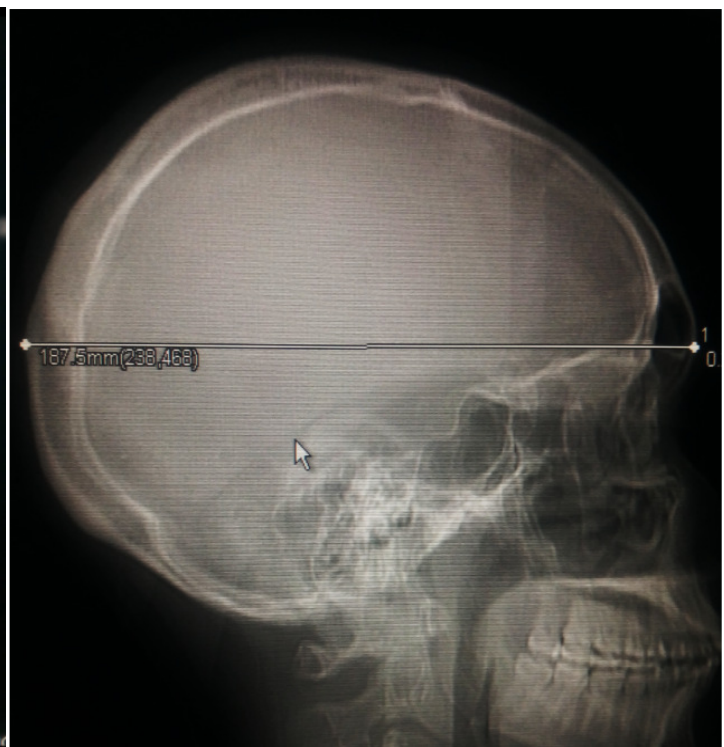


Fig. 1b: Lateral scanogram measuring cephalic length.

Cephalic width was measured as the maximum distance between the two euryons (lateral most point placed on the side of the head) on the AP-scanograms (Figure 1a). The cephalic length was measured as the maximum distance between the glabella (point above the nasal root between the eyebrows and intersected by mid-sagittal plane) and opisthocranon on the lateral scanograms (Figure 1b).^[20] Each measurement (in millimeters) was done

three times by one of the researchers and the average recorded. The reliability of the measurements was assessed using Intra-class Correlation Coefficient (ICC). Three separate measurements of randomly selected twenty-five CT series in 3 different days and a Cronbach alpha of 0.86 was obtained. Subsequently, Cephalic index (CI) was determined by the following formula:

CI= Cephalic width(mm) $\times$ 100/ Cephalic length(mm).^[2,3]

The classification of the participants' head type was based on the internationally recognized Martin-Saller scale.^[2,7,20,21,23-29] Data collected was analysed, SPSS Statistics, version 20.0. Mean, standard deviation (SD) and percentage were used to express data. Independent samples t-test was used to

determine differences in study parameter between sexes. A *P*-value of 0.05 was used as level of significance.

Results

The overall mean of cephalic length (CL) and cephalic width (CW) in the present study was determined to be 189.5mm $\pm$ 3.86 and 129.7 mm $\pm$ 2.52 respectively. The mean of CL and CW was further determined based on sex as seen in Table 1.

Table 1: Mean and SD of cephalic height and width according to sex

Anthropometric Parameters	Min -Max	Males (n=100) Mean $\pm$ SD	Females (n=53) Mean $\pm$ SD
Cephalic length(mm)	180-197	189.90 $\pm$ 3.91	188.7 $\pm$ 3.68
Cephalic width(mm)	122-135	129.60 $\pm$ 3.38	129.8 $\pm$ 3.94

The mean cephalic index (CI) for the study population was determined to be 68.46 $\pm$ 2.29. Furthermore, CI based on gender was also determined in Table 2.

Table 2: Cephalic index distribution index of males and females

Variable	Gender	Mean $\pm$ SD	Minimum	Maximum
Cephalic Index	Males	68.3 $\pm$ 2.542	63.60	75.00
	Females	68.77 $\pm$ 1.72	65.60	72.20

Based on Martin-Saller scale, the distribution of head shapes in the study for both males and females were also found to be dominantly dolicocephalic [n=150 (98%)] and fewer mesocephalic [n=3 (3%)] categories as shown in Table 3.

Table 3: Distribution of head shapes among sex.

Head shapes	Frequency (%)	
	Males	Females
Dolicocephalic	97(97.0%)	53(100%)
Mesocephalic	3(3.0%)	0(0%)
Total	100	53

The present study could not establish the existence of sexual dimorphism in CI among Hausa-fulani

population as shown in Table 4.

Table 4: Sexual dimorphism in CI between the sexes

Cephalic Index	t-test	Df	p-value
CI males +females	268.69	49	0.08

Discussion

Ethnicity is a variable that affects craniofacial dimensions.^[1] The cephalic index is an important parameter for deciding the race and sex of an individual whose identity is unknown.^[21] The mean

CL and CW of the present study are lower than what was reported by Umar *et al.* in 2006^[7] and Adebisi in 2003^[22] who worked on Hausa-Fulani in Jos, North-central Nigeria and Zaria, North-west Nigeria.

The difference in mean values could be attributable to a difference in method of measurement of variables (CL and CW) among the studies. Umar *et al* [7] and Adebisi [22] utilised external callipers in measurement.

The use of external callipers accommodates participant's differences and may be subject to variation in the measurement. Fat distribution in subcutaneous tissues differs between obese and normal participants even in the head, as Wang *et al.*, in 2014 reported a strong relationship between obesity and head fat.[23] Further, socio-cultural practices especially among the Hausa-Fulani where plating of hair is a customary culture and the religious practice of wearing veil (niqaf) over the head among females in the study area may account for these higher mean values reported by Umar *et al* [7] and Adebisi. [22] However, scanograms eliminate all these possibilities by measuring the external cortical surface of bony landmarks. Hence promising to be more objective and reflective of actual measurements of the variables.

The present study's CI when compared to the international standard classification of head types, falls within the dolicocephalic head type for both genders.[2,23] Similar findings have been reported by Maina *et al.*, in 2012.[24] They worked on Hausa-Fulani tribe from Gombe, North-eastern Nigeria, and reported a CI of 71.40 and also with the works of Jervas *et al.*, in 2016 that worked in a dominantly Igbo tribe population in Owerri, South-eastern Nigeria who reported CI of 71.57 ± 14.81 . [25] Both studies established values falling under the dolicocephalic head shapes. This means that some similarities exist between Hausa-Fulani and Igbo head shapes. However, their reported values appeared slightly higher than what is obtained in the present study. The difference might be as a result of the earlier highlighted reason for differences in methodology among the studies concerned. Similarities in findings of head shape between the present study and the works of Jarvas *et al.*, in 2016 may mean that geographical factors may have little influence on the head shapes of both populations despite Hausa-Fulani living in tropics and Igbos living in temperate zones. This fact is however not in agreement with studies reported by Oladipo and Anugweje in 2014 among Yoruba ethnic groups,

Bharati *et al.* in 2001 among Indian population and Garba *et al.*, in 2008, in Maiduguri.[26-28] They all reported that tropical zones head form is usually longer (dolicocephalic) while temperate zones head form is more round (mesocephalic or brachycephalic). The reason for these differences may be attributed to sample size differences and the observed wide standard deviation from mean CI in all the studies. Anthropological studies based on racial changes have determined that people from Africa, India, Australia, central part of Europe and North America are dolicocephalic.[2,3] The head shapes of peoples in the Pacific Ocean are of the brachycephalic type, while in the Middle East,

Russia and the central part of Europe are mesocephalic and most people living along the borders of the Atlantic Ocean are of the mesocephalic type.[2,3] Variations in the head shapes of different populations can be due to a complex interaction between genetic and environmental factors.[2,22] Moreover, ecological, biological, racial, gender and age factors have been reported to influence bodily dimensions.[5] However, similar works by Adebisi in 2003 [22] and Umar *et al.*, in 2011 [3] reported mean CI of Hausa-Fulani to be 75.89 and 76.7 respectively.[3,20] These studies qualified the Hausa-Fulani into mesocephalic head form and in contrast with the present study and that of Maina *et al.* 2015.[24] Differences can be regarded due to study location, sample size and methodology differences among the studies. The present study did not establish any statistically related sex-related differences in CI ($p > 0.05$). Few works like that of Jervas *et al.* in 2016 that worked on Igbo tribe in southeast Nigeria reported a similar finding. [25] It also conforms to the works of Lobo *et al.* in 2005 that worked on the CI of Gurung community of Nepal.[29]

This implies that CI between sexes in some population may be similar irrespective of location and ethnicity. Studies of this nature requires larger sample size. The smaller sample size used may affect the generalization of our findings. However, based ethical reasons as it pertains to radiation protection, the researchers could only recruit available CT-series that satisfied the inclusion criteria.

Conclusion

Cephalic index and head type (dolicocephalic) of Hausa-Fulani in Kano were determined using CT head scanograms. A shift in methodology with high level of reproducibility. Findings of this will be of significance to future researchers, forensic scientists, rhinoplastic surgeons, anatomists, and anthropologists especially now that the study established no sex differences exist within this population.

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Recommendations

This newer method is recommended to be used for other tribes and a comparative analysis be made.

Declaration Of Conflict Of Interest

We (authors) hereby declare that there is no conflict of interest in the entire manuscript.

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Evaluation of the Effect of Heat Processing Methods on Some Nutrient Contents of Yellow and White Colour Varieties of *Sorghum Bicolor* (Sorghum)

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Abstract

Heat processing methods of food have been developed over the centuries and are adopted apparently to improve the quality of the food and for the inactivation of anti-nutritional factors that are heat-labile. During heating, certain nutrients of food may be altered or destroyed, while others might be improved. This research was aimed at evaluating the effect of heat processing methods on some mineral elements (Se, Zn, Fe, Mn, Mg, Cu, Ca, Na, K, and P) and vitamins (B₁, B₃ and B₆) content of yellow and white colour varieties of *Sorghum bicolor*. Grain samples were divided into four portions and were separately roasted, boiled and steamed. The unprocessed portion served as the control. The unprocessed and processed sorghum varieties were milled and subjected to mineral elemental and vitamins analysis using standard techniques. The results showed that the boiling, roasting and steaming caused significant reductions ($P < 0.05$) in the Fe, Mg, Ca and P contents of all the grains. Maximum loss was observed in the boiled sample while the minimum loss was observed in roasted samples. Roasting caused a significant increase ($P < 0.05$) in the concentration of Mn, Ca, Na and K. The concentration of the vitamins were significantly decreased ($P < 0.05$) in all the treatments with boiling causing the highest reduction and very little loss was observed after steaming. It could be inferred from the study that steaming is a better processing method in retaining vitamins content while roasting is a better option in retaining the mineral content of yellow and white *S. bicolor* varieties.

Keywords: *Sorghum bicolor*, Boiling, Roasting, Steaming, Mineral Elements.

Introduction

All human beings require sufficient food for growth, development and to lead an active and healthy life and these depend upon the quality and quantity of foodstuff one is able to include in a regular diet. The quality of food depends upon the presence of a relative concentration of various nutrients such as proteins, fat, carbohydrate, vitamins, and mineral. Plant materials form a major portion of their diet as such, their nutritive value is important. Nutrition plays a critical role in wellness, by not only providing essential nutrients but also promoting good health and preventing diseases.^[1] Nearly all foods, whether derived from plant or animal sources, are rarely fit for direct consumption and require some prior processing. Heat processing methods of food have been developed over the centuries and are adopted apparently to make the final product more attractive in flavor, appearance, taste, etc. ^[2] Heat processing methods have been around for a long time and continue today to play a fundamental role in daily life across the globe. ^[3]

Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crops; they are, therefore, staple food crops. They were amongst the first plants to be cultivated or domesticated. Cereals have been grown and used by man since ancient times. They have been the principal component of the human diet and have played a major role in shaping human civilization for thousands of years.^[4] Around the world, they are important staples and critical to daily survival of billions of people. More than 50% of world's daily caloric intake is derived directly from cereal grains consumption. ^[5]

Sorghum bicolor commonly known as sorghum or guinea corn is a large grain plant native to northern Africa. It is a member of the grass family *Poaceae* or *Gramineae* and is the world's fifth major cereal in terms of production. It is a staple food crop for millions of the poorest and most food-insecure

people in the semi-arid tropics of Africa, Asia, and Central America. [6] Sorghum grain contains 56–73% starch, 7-15% protein and 4-5% fat. [7] The highest amount of starch and protein occur in the endosperm. [8]

Sorghum is considered to be a “powerhouse of nutrition”. Some varieties are rich in antioxidants (e.g. tannins, found particularly in red-grained types, contain antioxidants that protect against cell damage, a major cause of diseases and aging) and all varieties are gluten-free, offering an alternative for those who suffer from wheat allergies. Sorghum is an excellent source of B complex vitamins riboflavin, Vitamin B₆, thiamin, niacin (in small quantity) and minerals such as iron, potassium, manganese, zinc, phosphorus, and magnesium. [1] In fact, sorghum has been reported to be a good source of more than 20 micronutrients. It contains phytochemicals including lignans, phenolic acids, phytic acid, plant sterols, and saponins. [8] Seeds of sorghum can occur in white, creamy, yellow, pink, brown or violet colour. [9] Based on the ongoing discussion, it is necessary to assess the effect of traditional heat processing methods of our local foods. Hence, this research work was aimed at evaluating the effect of some heat processing methods of millet on some of its nutrient contents.

Materials and Methods

Chemical Reagents used

All the chemical reagents used were of analytical grade.

Equipment Some of the equipment used include: HPLC machine (AGILENT 1260 Infinity), Microwave Plasma Atomic Emission Spectrometer (MP-AES AGILENT 4200), flame photometer (Jenway PFP7), spectrophotometer and digital weighing balance.

Collection of Plant Material

The yellow and white *Sorghum bicolor* samples were obtained from local farmers in Bunkure Local Government Area, Kano State, and were authenticated in the Department of Plant Science Bayero University Kano, with Herbarium Accession Number BUKHAN 0475.

Sample Preparation

Grain samples were divided into four portions each,

crushed and three of the four portions were separately processed by boiling, roasting and steaming. The unprocessed sample served as control. a. The boiling process was done by suspending 200g of the crushed sorghum varieties in distilled water in the ratio 1:4 (w/v) for exactly 50 minutes at 100 – 140 °C until almost all the water was absorbed, the remaining leftover was drained off.

b. Roasting was done at a temperature of 110 °C by constantly stirring 200g of the sorghum varieties to prevent charring in a dry aluminum pan until browned.

c. Steaming was done by suspending 200g of each sorghum sample over 1 liter of boiling distilled water at 100 – 110 °C for 60 minutes.

The roasted sorghum was exposed to air to hasten cooling, while the boiled and steamed ones were oven-dried at 50 °C for four hours, ground and stored in an airtight glass jar at room temperature (~25 °C) prior to analysis. [10]

Digestion of Sample:

Exactly 1.0g of the powdered sample was taken in a crucible and placed into a muffle furnace, ashed at 500 °C and then cooled. Three ml of concentrated HCl was added and evaporated to dryness. Twenty ml of 25% HCl was then added to the residue to extract the mineral. The extract was transferred to 100ml volumetric flask and the volume made up to 50ml with distilled water. The digested solution was transferred to plastic bottles labeled accurately and was used mineral determination.

Determination of Mineral element

Analysis of Se, Fe, Zn, Mn, Cu and Ca content of the samples was carried out using microwave plasma atomic emission spectrometry (MP-AES). Sodium and potassium were determined using flame photometry while phosphorous using the phosphomolybdate method. The analysis was carried out after wet digestion of the sample. [11]

Determination of Phosphorus (P) by Spectrophotometry

Phosphorus in the sample was determined in accordance with the method described by AOAC. [11]

Vitamins Determination

Vitamins B₁, B₃, and B₆ of the samples were quantified using the methods described by Roqayya *et al.* [12]. The vitamins were first extracted from the sample before being injected into the HPLC machine. The first step of the extraction involves the liberation of vitamin and vitamin phosphate esters from their association with proteins through acid hydrolysis under high temperature. The sample was then subjected to enzyme hydrolysis to convert the phosphorylated vitamin forms into the free forms.

Statistical Analysis

The data obtained were presented as mean $\pm$ standard deviation. The data were analysed using one-way Analysis of Variance (ANOVA) followed

by Post Hoc (Least significant difference test). The results were considered statistically significant at $p < 0.05$.

Results

Minerals Content of Yellow and White *Sorghum bicolor* The results for mineral elemental contents of unprocessed and processed yellow and white *S. bicolor* are presented in Table 1. The levels of selenium, iron, magnesium, manganese, calcium, and phosphorus were significantly reduced ($P < 0.05$) as compared to control in all the treatments, while sodium level was significantly increased in processed samples. A significant reduction ($P < 0.05$) was observed in potassium levels of all the processed yellow *S. bicolor* as compared to control.

Table 1: Effect of heat processing methods on the mineral contents of yellow and white varieties of *Sorghum bicolor*

Mineral Elements (mg/Kg)	<i>S.bicolor</i> (Yellow)				<i>S.bicolor</i> (White)			
	Raw	Boiled	Roasted	Steamed	Raw	Boiled	Roasted	Steamed
Se	1.91 $\pm$ 0.00 ^a	1.07 $\pm$ 0.00 ^a	1.81 $\pm$ 0.00 ^a	1.42 $\pm$ 0.00 ^a	1.50 $\pm$ 0.01 ^{ac}	1.51 $\pm$ 0.00 ^{bd}	1.68 $\pm$ 0.05 ^{acd}	1.56 $\pm$ 0.00 ^{acd}
Zn	0.13 $\pm$ 0.00 ^{acd}	0.13 $\pm$ 0.00 ^{bcd}	0.10 $\pm$ 0.00 ^{acd}	0.14 $\pm$ 0.00 ^{acd}	0.11 $\pm$ 0.00 ^a	0.14 $\pm$ 0.00 ^a	0.11 $\pm$ 0.00 ^a	0.12 $\pm$ 0.00 ^a
Fe	10.41 $\pm$ 0.00 ^a	1.26 $\pm$ 0.01 ^a	1.14 $\pm$ 0.00 ^a	1.32 $\pm$ 0.00 ^a	6.61 $\pm$ 0.00 ^a	1.07 $\pm$ 0.02 ^a	2.36 $\pm$ 0.03 ^a	1.56 $\pm$ 0.03 ^a
Cu	0.03 $\pm$ 0.00 ^{ac}	0.04 $\pm$ 0.00 ^{ac}	0.03 $\pm$ 0.00 ^c	0.03 $\pm$ 0.00 ^c	0.03 $\pm$ 0.00 ^{ac}	0.03 $\pm$ 0.00 ^{ac}	0.03 $\pm$ 0.00 ^{ad}	0.02 $\pm$ 0.00 ^{bc}
Mg	26.41 $\pm$ 0.00 ^a	23.69 $\pm$ 0.00 ^a	24.39 $\pm$ 0.00 ^a	25.65 $\pm$ 0.00 ^a	23.49 $\pm$ 0.00 ^a	20.92 $\pm$ 0.01 ^a	22.90 $\pm$ 0.02 ^a	22.20 $\pm$ 0.01 ^a
Mn	0.25 $\pm$ 0.00 ^a	0.17 $\pm$ 0.00 ^a	0.20 $\pm$ 0.00 ^a	0.20 $\pm$ 0.00 ^a	0.21 $\pm$ 0.00 ^a	0.18 $\pm$ 0.00 ^a	0.20 $\pm$ 0.00 ^a	0.18 $\pm$ 0.00 ^a
Ca	299.22 $\pm$ 0.09 ^b	240.84 $\pm$ 0.09 ^b	268.90 $\pm$ 0.09 ^b	145.22 $\pm$ 0.01 ^b	259.28 $\pm$ 0.05 ^b	210.23 $\pm$ 0.04 ^b	216.48 $\pm$ 0.09 ^b	29.00 $\pm$ 0.01 ^b
K	1.60 $\pm$ 0.01 ^b	1.30 $\pm$ 0.01 ^b	1.40 $\pm$ 0.01 ^b	1.50 $\pm$ 0.01 ^b	0.81 $\pm$ 0.01 ^b	0.80 $\pm$ 0.01 ^a	0.81 $\pm$ 0.01 ^c	0.70 $\pm$ 0.01 ^{bac}
Na	2.40 $\pm$ 0.01 ^b	2.92 $\pm$ 0.02 ^b	2.60 $\pm$ 0.01 ^b	2.41 $\pm$ 0.01 ^b	2.01 $\pm$ 0.01 ^b	2.20 $\pm$ 0.01 ^b	2.12 $\pm$ 0.02 ^b	3.01 $\pm$ 0.01 ^b
P	625.63 $\pm$ 0.71 ^c	227.33 $\pm$ 0.29 ^c	397.50 $\pm$ 0.50 ^c	454.00 $\pm$ 0.50 ^c	567.83 $\pm$ 0.29 ^c	284.13 $\pm$ 0.32 ^c	341.00 $\pm$ 0.50 ^c	301.8 $\pm$ 0.26 ^c

Values are expressed as mean $\pm$ standard deviation.

Means with the same superscript in the same row are significantly different at $P < 0.05$ following LSD Post Hoc.

Vitamins B₁, B₃ and B₆ Content of Unprocessed and Processed Yellow and White *Sorghum bicolor*

The results obtained for vitamin B₁ content of *S. bicolor* varieties are shown in Figure 1. The yellow variety has the highest vitamin B₁ content while the white variety has the lowest content. The vitamin B₁ contents of all the samples were significantly ($P < 0.05$) reduced after processing with the exception of white *S. bicolor* where an insignificant increase

was observed after roasting. Among the treatments, boiling had the most adverse effect accounting for a loss of 26% in yellow *S. bicolor* and 23.4% of vitamin B₁ contents in white *S. bicolor*. Roasting also caused a loss of 11.5% of vitamin B₁ in yellow *S. bicolor* and steaming on the other hand caused minimal loss as compared to boiling and roasting. It caused a loss of 11.1% in yellow *S. bicolor* and 9.4% in white *S. bicolor*.

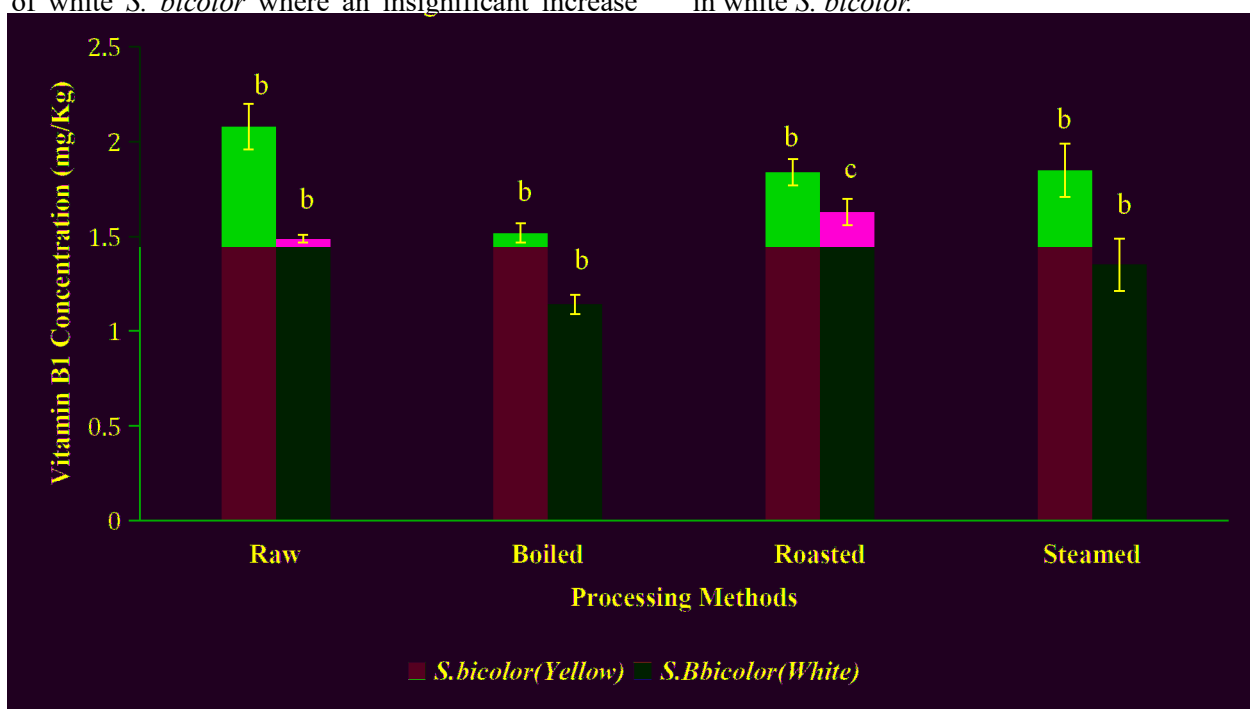


Fig. 1: Concentration of Vitamin B₁ of the raw and processed sorghum samples.

Values within the same group bearing similar superscript are significantly different at $P < 0.05$.

The results for the concentration of vitamin B₃ in the unprocessed and processed *S. bicolor* varieties are presented in Figure 2. Yellow *S. bicolor* had a higher content of vitamin B₃ than the white variety. The vitamin B₃ content was significantly ($P < 0.05$) reduced in all the treatments. The boiling method

exhibited highest adverse effect accounting for a loss of 32.2% in yellow *S. bicolor* and 54.7% in white *S. bicolor*. The steaming method caused a little loss of 22.9% in yellow *S. bicolor* and 19.8% of vitamin B₃ content in white *S. bicolor*.

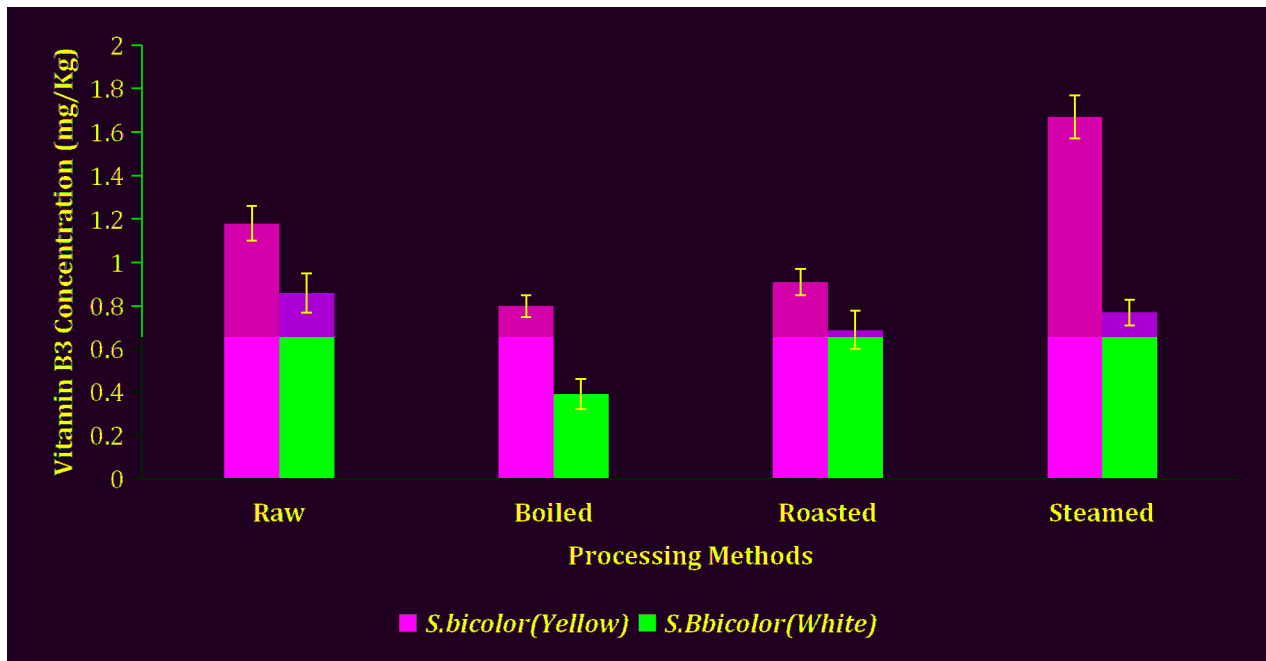


Fig. 2: Concentration of Vitamin B₃ in unprocessed and processed sorghum bicolor varieties.

The results obtained for the concentration of vitamin B₆ in the unprocessed and processed *S. bicolor* varieties are shown in Figure 3. The yellow variety has higher content than the white one. Boiling caused the highest loss of vitamin B₆. It caused a

loss of 27.2% in yellow *S. bicolor* and 26.4% in white *S. bicolor*. Steaming also caused a loss of 25.3% in yellow *S. bicolor* and 10.9% in white *S. bicolor*. Maximum retention of vitamin B₆ was observed in the roasted samples.

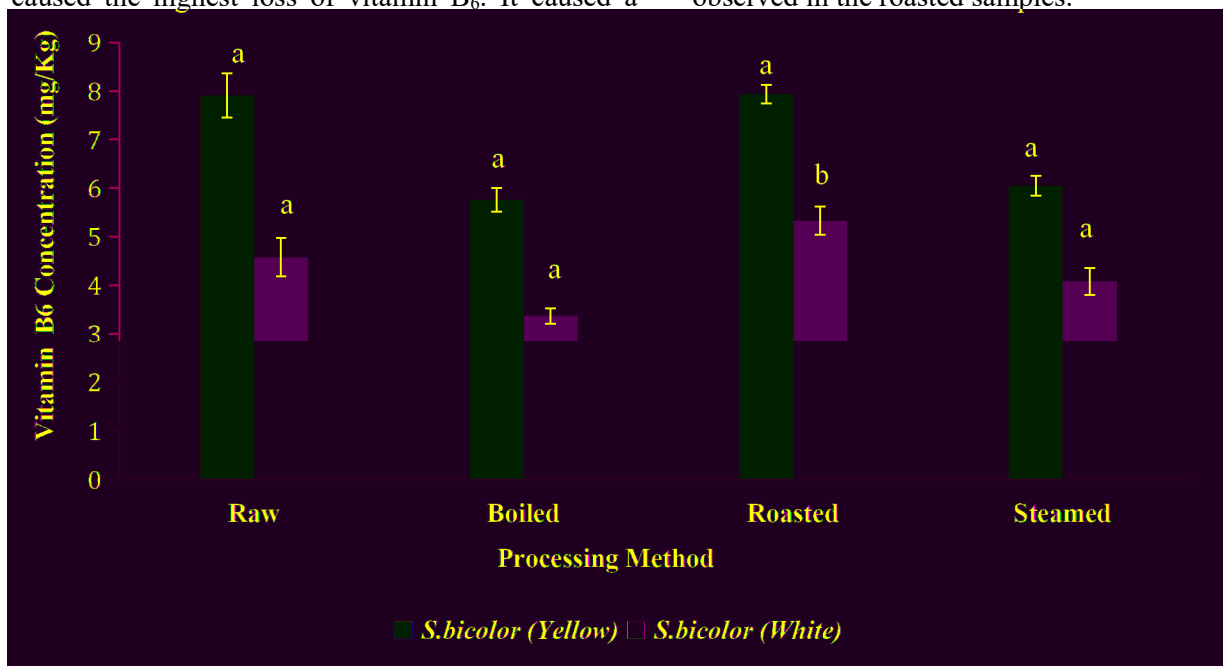


Fig. 3: Concentration of Vitamin B₆ in Raw and processed *Sorghum bicolor* Varieties.

Values within the same group bearing similar superscript are significantly different at $P < 0.05$.

Discussion

Sorghum bicolor being a powerhouse of nutrition contributes nutritionally to protein, mineral and vitamin intakes. The results obtained from the study indicated that they were rich in P, Mg, Fe and Ca. The good availability of calcium, magnesium, and phosphorus is a good indication that *S. bicolor* is rich in the minerals for bone formation. During heating, certain sensitive nutrients may alter or destroy while others might improve. From the results of this study, boiling caused the highest loss of mineral elements. This substantial reduction may be due to solubility which causes leaching of the minerals into the boiling water and also may be related to the large volume of water used.^[13] However, roasting method caused an increase in calcium, zinc, manganese and sodium contents of the studied samples.. This may be due to decrease in the water content thereby causing the changes associated with dehydration such as concentrating these minerals in the roasted samples.^[14] The observed minimal decrease in the mineral content of the steamed grains may also be due to volatility as some of the volatile minerals escaped through the steam.^[15] These results are in good agreement with those reported by ^[16] who reported that steaming processes decreased the mineral content of raw sunflower seeds by about 39.2%. A decrease in minerals was also observed by ^[17] after cooking (boiling and roasting) which is attributed mainly due to enhanced permeability of seed coat of underutilized legumes of Nigeria.

Heat processing is aimed primarily at increasing the palatability of food. Nutrients that are water-soluble, for example vitamins like vitamin C and vitamin B and a group of nutrients called polyphenols seem to be the most vulnerable to degradation in cooking. From the results of this study, there is a general decrease in the vitamin B₁, B₃ and B₆ content of the heat-processed sorghum. Vitamin B₁ and B₆ contents were affected to some extent depending not only on the type of method of processing but also the variety of sorghum used. This may be due to differences in the varieties used or processing time.^[18] The reduced vitamin content of the processed sorghum could be attributed to the severity of the heat during processing and a reflection of the instability of the vitamins to heat.^[19] Several researchers reported that all the B vitamins are sensitive to heat, but

thiamin, also known as vitamin B₁, and cobalamin or vitamin B₁₂, are the most unstable and likely to be destroyed by cooking. Others reported that B₆ and B₁₂ are unstable.^[20] Vitamin B₁ content of rice cooked using various methods of cooking were studied.

Thiamine was significantly reduced in each method. This may be due to partial degradation of the vitamin when subjected to heat.^[21] The significant increase in the vitamin content in the roasted white *S. bicolor* may be that the vitamin is incompletely extracted in the raw grain before processing.^[22] Vitamin B₃ is more stable (not easily affected by heat, light, and oxygen) than most other B vitamins though it is highly soluble in water. It has been reported to be hardly affected by cooking. In another study conducted ^[23] no significant reduction in the vitamin B₃ content of some selected vegetables was recorded, which was due to its stability. This is not in accordance with the findings of this research and maybe as a result different conditions used such as processing time and temperature. The findings of this research is in accordance with the findings of ^[24] who reported a significant decrease in vitamin B₃ contents of fish cooked by different methods. This loss may be due to leaching and prolonged cooking time. The results of this study are also in accordance with the findings of ^[25] who studied the effect of cooking methods on the mineral and vitamin contents of African catfish. In a study conducted ^[26] to evaluate the impact of heat treatment on water-soluble vitamins in milk, it was observed that boiling for 15 minutes caused a depletion of vitamins B₁, B₂, B₃, B₆ and folate by 24 to 36 percent. Prolonged exposure of vitamin B₆ to heat can degrade it in most foods. Perhaps as a result of structural difference, it is degraded most in animal than plant foods.^[27] It was reported ^[28] that both steaming and boiling result in relatively low amounts of vitamin B₆ loss. It showed that boiling resulted in greater loss of the vitamin in Brussels, presumably because of submersion in water allowing more surfaces to water contact. Also, in a study reported ^[26] boiling milk for 15 minutes caused depletion of vitamins B₁, B₂, B₃, B₆ and folate by 24 to 36 percent.

These findings are consistent with the results of this study. In a study conducted ^[29] to determine the effect of boiling, roasting, steaming and stir-frying on vitamin B₁ and B₆ in foods observed that the three methods caused a significant decrease of the vitamins by a percentage of 35-60% in boiling, 10-40% in roasting and 10-25% in steaming. Generally, the decrease in the concentration of these water-soluble vitamins in the boiled samples may be due to leaching (Boiling dissolves and washes the vitamins from the grains) into the boiling water. It was also reported ^[29] that water-soluble vitamins leak from foods when they come in contact with water but the losses become significant when heat is applied. They are mostly destroyed by moist heat methods of processing including steaming but to a greater extent in boiling. The decrease in steaming and roasting

may be due to thermal decomposition. Steaming causes minimal loss, this may be due to less contact with water and so the vitamins are slightly affected by the high temperature of the cooking medium. The decrease of the vitamins in the roasted sample may be due to direct contact with heat. ^[30]

Conclusion

The results of the present study showed that all the heat processing methods affected the nutrient contents of the yellow and white *Sorghum bicolor*. Boiling exhibited a more deleterious effect while steaming and roasting are better. Steaming retained most of the vitamins while roasting retained most of the mineral elements.

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Effect of Different Heat Processing Methods on Some Nutrient Contents of Two Varieties of *Pennisetum Glaucum* (Pearl Millet)

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Abstract

Heat processing methods have been around for a long time and play a fundamental role in daily life, globally. Heat processing methods can degrade some nutrients and can enhance the availability of others. This research work was aimed at assessing effect of heat processing methods on some the mineral elements (Se, Zn, Fe, Mn, Mg, Cu, Ca, Na, K, and P), and some vitamins (B₁, B₃, and B₆) contents of two varieties of *Pennisetum glaucum* (*Pennisetum typhoides* and *Pennisetum maiwa*) Millet samples were divided into four portions each and were separately roasted, boiled and steamed. The unprocessed portion served as the control. The unprocessed and processed grains were milled and subjected to mineral elemental and vitamin analysis. The results showed that the Boiling, roasting and steaming caused significant reductions ($P < 0.05$) in the levels of Fe, Mg, Ca and P of all the studied grains. Maximum loss was observed in the boiled sample while minimum loss was observed in roasted samples. Roasting caused a significant increase ($P < 0.05$) in the concentration of Mn, Ca, Na and K. The concentration of the vitamins were significantly decreased ($P < 0.05$) in all the treatments with boiling causing the highest reduction level and very little loss was observed after steaming. The results of this study revealed that steaming is a better processing method in retaining the vitamins levels while roasting is a better option in retaining the mineral elements content of both *P. glaucum* varieties.

Keywords: *Pennisetum glaucum*, Vitamins, Mineral elements, Roasting, Steaming.

Introduction

Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crops; they are, therefore, staple food crops. They were amongst the first plants to be cultivated or domesticated. They have been grown and used by man since ancient times. Cereals have been the principal component of human diet and have played a major role in shaping human civilisation for thousands of years. [1] Around the world, cereal grains are important staples and critical to daily survival of billions of people. More than 50% of world's daily caloric intake is derived directly from cereal grains consumption.[2] Some researchers believe that heat processing methods were invented over 2.3 million years ago, whilst others argue it is a more recent concept, being invented only 40,000 years ago. Despite these opposing views, it is clear that heat processing methods have been around for a long time and continues today to play a fundamental role in daily life across the globe. [3] Several studies have shown that while heat processing methods can degrade some nutrients, it can enhance the availability of others. Traditionally, cereals are

usually boiled, steamed or roasted to improve its eating quality before consumption. [4]

Pennisetum glaucum (pearl millets) are small-grained, annual, warm-weather cereals belonging to grass family. They are important crops in the semi-arid tropics of Asia and Africa, especially in India, Mali, Nigeria, and Niger. It is called *gero* in Hausa, *arumin* in Borno Kanuri, and *oka* in Yoruba. [5] Its consumption has been practiced since the beginning of the ancient civilizations of the world. More than 90 million desperately poor people depend on pearl millet for food [6] It is nutritious compared to the major cereals such as rice and wheat. [7] Pearl millet is a good source of protein, carbohydrates, B vitamins and minerals.[8] It has low levels of lipids and diverse health-promoting phenolic compounds.[9] Pearl millet types in Nigeria differ so much that short duration millet was classified as *Pennisetum typhoides* while long-duration millet as *Pennisetum maiwa*. [10] They are rarely eaten raw and are usually processed by various cooking methods, such as boiling, roasting and steaming before consumption.

These heating processes are applied to enhance flavor and taste of food and inactivate pathogenic microorganisms. [11]

Considering the above, it is imperative to conduct researches in order to assess the effect of different heat processing methods on the quality of our local foods. Hence, this study was aimed at evaluating the effect of some heat processing methods on some nutrient contents of two varieties of *Pennisetum glaucum*.

Materials and Methods

Chemical Reagents used

All the chemical reagents used were of analytical grade.

Equipment

Some of the equipment used include: HPLC machine (AGILENT 1260 Infinity), Microwave Plasma Atomic Emission Spectrometer (MP-AES AGILENT 4200), flame photometer (Jenway PFP7), spectrophotometer and digital weighing balance.

Collection of Plant Materials

Pennisetum typhoides and *Pennisetum maiwa* samples were obtained from local farmers in Bunkure Local Government Area, Kano State, and were authenticated in the Department of Plant Science, Bayero University, Kano, with Herbarium Accession Number BUKHAN 0249.

Sample Preparation

The millets were divided into four portions each, crushed and three of the four portions were processed differently. [12] The different processing methods include; boiling, roasting and steaming. The portion that was not processed with the above method served as the control.

a. The boiling process was done by suspending 200g of the crushed whole millets in distilled water in the ratio 1:2.5(w/v) for exactly 20minutes at 100 – 140 °C until almost all the water is absorbed, the remaining left was drained.

b. Roasting was done at a temperature 110 °C by constantly stirring 200g each of the millets separately to prevent charring in a dry aluminum pan until browned.

c. Steaming was done by suspending 200g of each of the millet over 1 litre of boiling distilled water at 100 –110 °C for 30 minutes.

The roasted millet was exposed to air to hasten cooling, while the boiled and steamed ones were oven-dried at 50 °C for four hours, grounded and stored in an airtight glass jar at room temperature (~25 °C) prior to analysis.

Sample Digestion

Exactly 1.0g of the powdered sample was taken in a crucible and placed into a muffle furnace, ashed at 500 °C and then cooled. Three ml of concentrated HCL was added and evaporated to dryness. Twenty cm³ 25% HCL was then added to the residue to extract the mineral. The extract was transferred to 100cm³ volumetric flask and the volume made up to 50cm³ with distilled water. The digested solution was transferred to plastic bottles labeled accurately and was used mineral determination.

Determination of Mineral elements

Analysis of Se, Fe, Zn, Mn, Cu and Ca content of the samples was carried out using microwave plasma atomic emission spectrometry (MP-AES). Sodium and potassium were determined using flame photometry while phosphorous using the phosphomolybdate method. The analysis was carried out after wet digestion of the sample. [13]

Determination of Phosphorus (P) by Spectrophotometry:

The phosphorus in the sample was determined according to the method described by AOAC. [13]

Vitamin Determination

The vitamins were first extracted from the sample before being injected into the HPLC machine. The first step of the extraction involves the liberation of vitamin and vitamin phosphate esters from their association with proteins through acid hydrolysis under high temperature. The sample was then subjected to enzyme hydrolysis to convert the phosphorylated vitamin forms into the free forms. [14]

Statistical Analysis

The data obtained were presented as mean ± standard deviation. The data were then analysed using one-way Analysis of Variance (ANOVA) followed by Post Hoc (least significant difference test). The results were considered statistically significant at p<0.05.

Results

Minerals Content of Unprocessed and Processed *Pennisetum glaucum* varieties The results obtained for the mineral elemental contents of raw (unprocessed) and processed *Pennisetum glaucum* varieties are presented in Table 1. Boiling, roasting and steaming caused significant reductions ($P<0.05$) in the Fe, Mg, Ca and P contents of all the studied grains as compared to control. Their concentrations

varied with treatments, with higher concentrations in the raw followed by the roasted then steamed samples. The boiled samples have the least mineral elemental contents while the maximum retention was observed in the roasted samples. Roasting also caused a significant increase ($p>0.05$) in Ca, Mn, Na, and K concentrations. Variable effects on the Se, Zn, Na and K contents of the processed grains were observed in both samples.

Table 1: Effect of heat processing methods on the mineral elemental contents of *Pennisetum maiwa* and *Pennisetum typhoides*

Mineral Elements (mg/Kg)	<i>Pennisetum maiwa</i>				<i>Pennisetum typhoides</i>			
	Raw	Boiled	Roasted	Steamed	Raw	Boiled	Roasted	Steamed
Se	1.77±0.01 ^a	1.32±0.00 ^a	1.41±0.00 ^a	1.35±0.01 ^a	1.20±0.00 ^{ac}	1.03±0.01 ^{ac}	1.57±0.00 ^{ac}	1.19±0.01 ^{bc}
Zn	0.19±0.01 ^c	0.12±0.00 ^c	0.16±0.00 ^c	0.15±0.00 ^c	0.33±0.00 ^{ab}	0.19±0.00 ^{ab}	0.20±0.00 ^{ac}	0.23±0.02 ^{ab}
Fe	38.36±0.01 ^a	1.47±0.21 ^a	8.42±0.00 ^a	5.73±0.00 ^a	18.72±0.00 ^a	1.34±0.00 ^a	2.68±0.00 ^a	1.46±0.03 ^a
Cu	0.14±0.18 ^a	0.04±0.00 ^b	0.04±0.00 ^c	0.04±0.00 ^d	0.03±0.00 ^{abcd}	0.03±0.00 ^b	0.03±0.00 ^c	0.03±0.00 ^d
Mg	26.33±0.01 ^a	23.69±0.01 ^a	24.46±0.05 ^a	23.77±0.00 ^a	26.65±0.01 ^a	23.67±0.01 ^a	24.45±0.00 ^a	24.17±0.01 ^a
Mn	0.23±0.00 ^a	0.25±0.00 ^a	0.29±0.00 ^a	0.30±0.00 ^a	0.16±0.00 ^a	0.31±0.00 ^a	0.20±0.00 ^a	0.18±0.00 ^a
Ca	118.58±0.21 ^b	101.74±0.04 ^b	105.67±0.06 ^b	34.34±0.09 ^b	44.27±0.07 ^b	42.26±0.00 ^b	143.78±0.21 ^b	33.19±0.005 ^b
K	1.50±0.01 ^b	1.4±0.01 ^b	1.6±0.01 ^b	1.2±0.01 ^b	1.01±0.01 ^{acd}	1.00±0.01 ^{be}	1.60±0.01 ^{cde}	1.50±0.01 ^{ad}
Na	2.51±0.02 ^a	3.11±0.01 ^a	3.20±0.01 ^a	2.57±0.06 ^a	3.24±0.05 ^b	2.47±0.06 ^b	2.61±0.02 ^b	3.11±0.01 ^b
P	1364.0±0.98 ^{ab}	284.13±0.32 ^{ab}	284.17±0.29 ^{ac}	909.07±0.40 ^{ab}	1875.±0.55 ^c	375.33±0.29 ^c	681.90±0.36 ^c	1193.17±0.29 ^c

Values are expressed as mean ± standard deviation (n=3).

Means with the same superscript in the same row are significantly different at $P<0.05$ following LSD Post Hoc.

Vitamin B₁, B₃ and B₆ Content of the Unprocessed and Processed *Pennisetum glaucum* Varieties

The results for the vitamin B₁ content of *Pennisetum glaucum* (*Pennisetum maiwa* and *Pennisetum typhoides*) are shown in Figure 1. *Pennisetum maiwa* exhibited higher vitamin content than *Pennisetum typhoides*. The vitamin B₁ content of all the samples was significantly ($P<0.05$) reduced by all the processing methods. Among the treatments, boiling

had the most adverse effect accounting for a loss of 19.4% in *Pennisetum typhoides* and 35.3% in *Pennisetum maiwa*. Roasting also caused a loss of 7.0% of the vitamin in *Pennisetum typhoides*, 31% in *Pennisetum maiwa*. Steaming on other hand caused a minimal loss as compared to boiling and roasting. It caused a loss of 4.7% in *Pennisetum typhoides* and 25.3% in *Pennnisetum maiwa*.

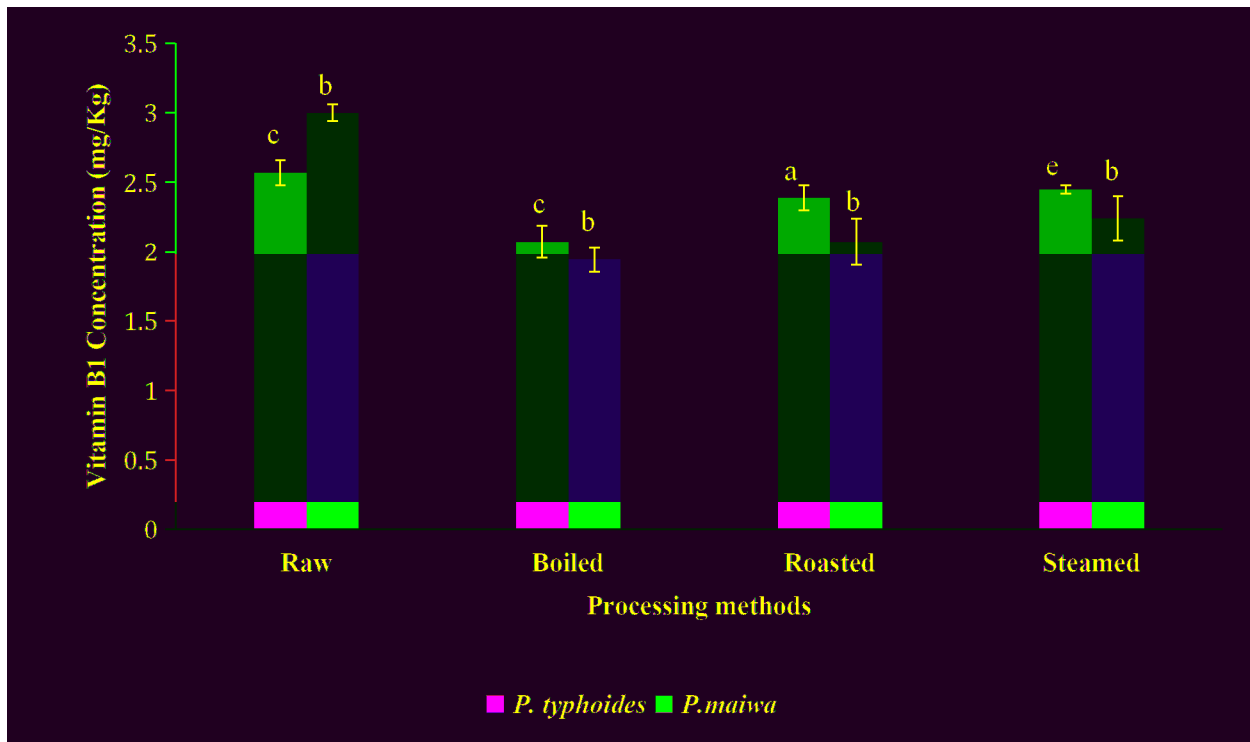


Fig. 1: Concentration of Vitamin B₁ of the raw and processed *Pennisetum glaucum* varieties. Values within the same group bearing similar superscript are significantly different at P<0.05.

The results obtained for the concentration of vitamin B₃ in the unprocessed and processed *Pennisetum glaucum* varieties are presented in Figure 2. *Pennisetum typhoides* had the highest content of the vitamin than *Pennisetum maiwa*. The vitamin content was significantly (P<0.05) reduced in all the treatments. Boiling had the most adverse effect accounting for a loss of 58.02% of the vitamin in

Pennisetum typhoides and 39.7% in *Pennisetum maiwa*, then roasting which caused a loss of 49.3% in *Pennisetum typhoides*, 24.45% in *Pennisetum maiwa*. Steaming caused a minimum loss as compared to boiling and roasting methods; it caused a loss of 27.81% in *Pennisetum glaucum* and 16.84% in *Pennisetum maiwa*.

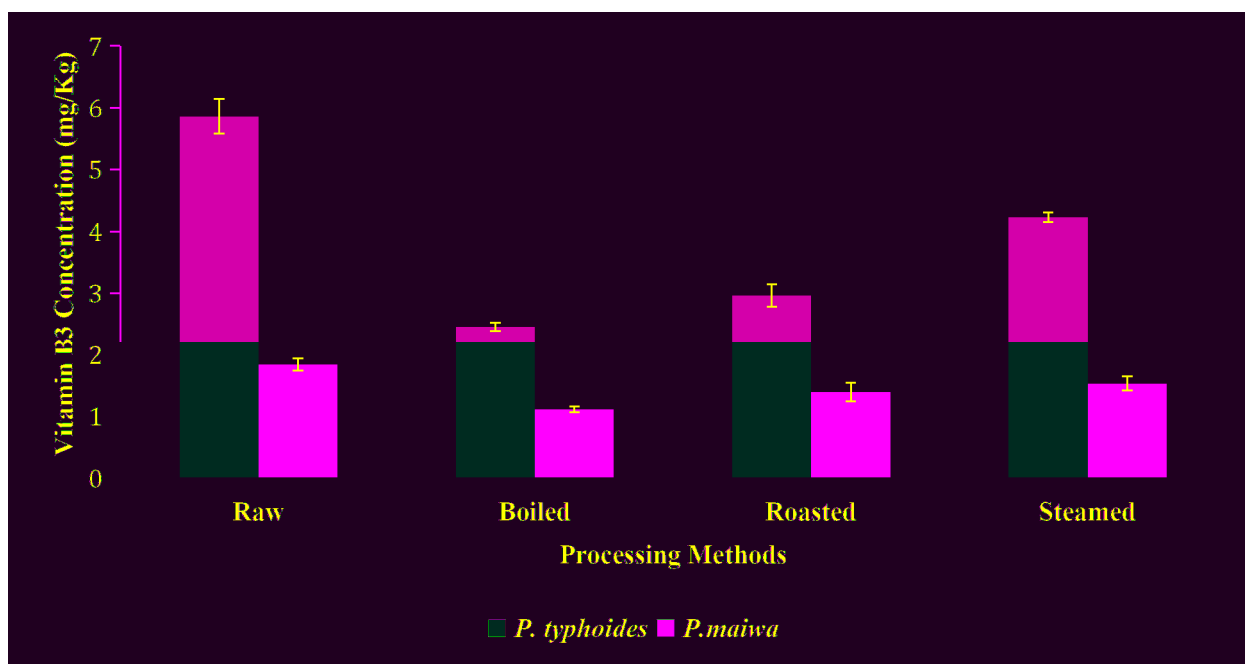


Fig. 2: Concentration of vitamin B₃ of the raw and processed *Pennisetum glaucuum* varieties. All values within the same group are significantly different at P<0.05.

The results for the concentrations of vitamin B₆ in the unprocessed and processed *Pennisetum glaucuum* varieties are shown in Figure 3. *Pennisetum typhoides* had the highest vitamin content than *pennisetum maiwa*. Boiling caused the highest loss of the vitamin, accounting for a loss of

15.7% in *Pennisetum typhoides* and 2.9% in *Pennisetum maiwa*. Steaming also caused a loss of 14.5% in *Pennisetum typhoides* and 16.4% in *Pennisetum maiwa* while maximum retention of the vitamin was observed in the roasted samples.

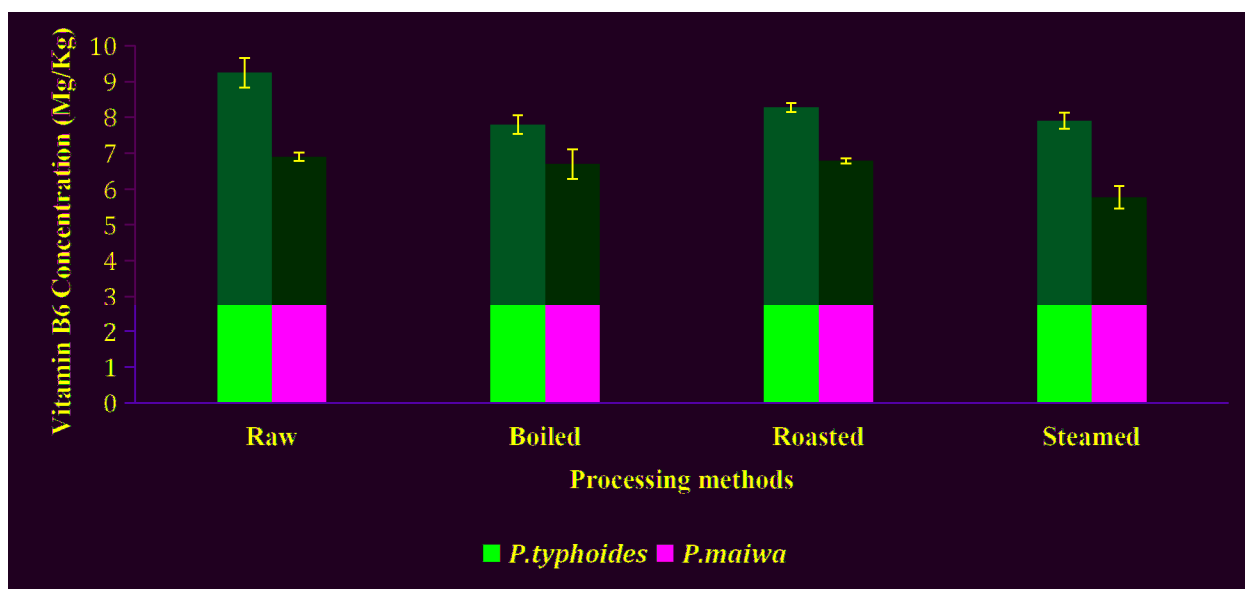


Fig 3: Concentration of vitamin B₆ in the in the unprocessed and processed *Pennisetum glaucuum* varieties (*Pennisetum typhoides* and *Pennisetum maiwa*). Values within the same group bearing similar superscript are significantly different at P<0.05.

Discussion

Pearl millets are important sources of food that contribute nutritionally to carbohydrate, protein, mineral and vitamin intake.^[15] The results indicated that P, Mg, Fe and Ca levels were generally high in both millet samples while the contents of Cu and Zn were very low. The good availability of calcium, magnesium, phosphorus is a good indication that the millets are so rich in the minerals for bone formation. During heating, certain sensitive nutrients may be altered or destroyed while others might improve. Nutrient loss is a general aftermath of various processing treatments. From the results of this study, boiling caused the highest loss of mineral elements. This substantial reduction may be due to the solubility which caused leaching of the minerals into the boiling water and also maybe related to the large volume of water used.^[16] The results of the study also revealed that roasting method increased the concentrations of calcium, zinc, manganese, and sodium of the grains. This may be due to decrease in the water content thereby causing the changes associated with dehydration such as concentrating these minerals in the roasted samples.^[17]

The observed minimal decrease in the mineral contents of the steamed grains may also be due to volatility as some of the volatile minerals escaped through the steam.^[18] These findings are in good agreement with those reported by Mariod *et al.*^[19] who reported that steaming processes decreased the mineral content of sunflower seeds by about 39.2%. A decrease in minerals was also observed by Aletor and Ojo^[20] after cooking (boiling and roasting) which is attributed mainly due to enhanced permeability of seed coat of underutilized legumes of Nigeria.

The results of this study also exhibited that the Vitamin B₁ and B₆ concentrations were affected to some extent, depending not only on the type of method of processing used but also the variety used. This may be due to differences in the varieties used or processing time.^[21] The reduction in vitamin contents of the processed millet samples observed in this study could be attributed to the severity of the heat during processing and a reflection of the instability of the vitamins to heat.^[22] Thiamine level was significantly reduced in each method and may be due to partial degradation of the vitamin when

subjected to heat.^[23] Vitamin B₃ is more stable (not easily affected by heat, light, and oxygen) than most other B vitamins though it is highly soluble in water. It has been reported to be hardly affected by cooking. Rumm-kreuter and Dammel^[24] recorded no significant reduction in the vitamin B₃ content of some selected vegetables which was due to its stability which disagreed with the findings of this research. However, the findings of this research is supported by the findings of Momenzadeh *et al.*^[25] who reported a significant decrease in vitamin B₃ contents of fish cooked by different methods. In the same vein, Leskova *et al.*^[26] reported that both steaming and boiling resulted in relatively low amounts of vitamin B₆ loss. It showed that boiling resulted in greater loss of the vitamin in Brussels, presumably because of submersion in water allowing more surfaces to water contact. Moreover, in a study conducted by Khair-un-nisa *et al.*^[27] boiling of milk for 15 minutes caused depletion of vitamins B₁, B₂, B₃, B₆ and folate contents by 24 to 36 percent. All of these findings are consistent with the results obtained in this study.

The observed decrease in the concentration of these water-soluble vitamins in the boiled samples may be due to leaching into the boiling water. Money^[28] reported that water-soluble vitamins leak from foods when they come in contact with water but the losses become significant when heat is applied. They are mostly destroyed by moist heat methods of processing including steaming but to a greater extent in boiling. The decrease in the levels of the vitamins in steaming and roasting may be due to thermal decomposition. Steaming caused minimal loss, this may be due to less contact with water and so the vitamins are slightly affected by the high temperature of the cooking medium. The decrease of the vitamins in the roasted sample may be due to direct contact with heat.^[29]

Conclusion

The results of the present study showed that all the heat processing methods affected the nutrient contents of the *Pennisetum typhoides* and *Pennisetum maiwa*. Boiling exhibited a more deleterious effect as compared to steaming and roasting methods of food processing. The steaming method retained most of the vitamins while roasting retained most of the mineral elements studied.

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The Role of Toll-Like Receptor-4 In the Production of TNF- α , IL-1 β and IL12P40 in Innate Immune Response to a Recombinant BCG-Based Malaria Vaccine Candidate

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Abstract

Our laboratory had earlier constructed a recombinant BCG expressing the MSP-1C of *Plasmodium falciparum* malaria vaccine candidate, which induced a robust innate immune responses leading the production of pro-inflammatory cytokines such as IL-1 β and TNF- α through a mechanism that had not been established but thought to involve toll-like receptor 4 (TLR-4) and its attachment to the vaccine candidate. In order to determine the role of the TLR-4 in eliciting innate immune responses against recombinant BCG expressing the MSP-1C of *P. falciparum* leading to the production of IL-1 β , TNF- α , and IL-12p40. Six groups of mice (n=6 per group) were immunised intraperitoneally with phosphate buffered saline T80 (PBS-T80), BCG or rBCG in the presence or absence of a TLR-4 inhibitor; TAK-242, given one hour prior to each immunisation. The mice were given a booster immunisation three weeks later and a second booster three weeks after the first. Peritoneal macrophages were harvested and cultured for determination of IL-1 β , TNF- α , and IL-12p40 in the supernatant, via ELISA. The results showed that the production of the three cytokines was significant in all mice groups, with the rBCG immunised group producing significantly higher cytokine than the two control groups. The production of the three cytokines was significantly inhibited in the presence of TAK-242 in all mice groups. The results obtained showed evidence of the role of TLR-4/rBCG attachment mechanism in eliciting innate immune responses and the increased production of IL-1 β , TNF- α , and IL-12p40, by our rBCG-based malaria vaccine candidate expressing MSP-1C of *P. falciparum*.

Keywords: Cytokine, Immunity, Malaria, MSP-1, Vaccine

Introduction

Malaria is a highly devastating, life-threatening disease caused by one or more of the four species of the *Plasmodium* genus: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* and the relatively newer, fifth form, *P. knowlesi*,^[1] introduced via the bite of the female *Anopheles* mosquito. Malaria leads to high morbidity and mortality in many parts of the world notably; Africa, South-East Asia and some parts of South America,^[2] where it is most devastating among children and pregnant women.^[3] Malaria control is based on the World Health Organisation (WHO) module of prevention, early diagnosis and prompt treatment of cases with drugs, the Artemisinin combination therapy (ACT).^[4] The efficiency of the module is threatened by insecticide resistance developed by *Anopheles* mosquito,^[5] and an increasing failure of the therapy ACT,^[6] by *P. falciparum* leading to its resistance to all antimalarials presently in use^[7] necessitating an intensified search for an effective antimalarial

vaccine,^[8] which include the use of BCG as a vector,^[9] expressing agonists such as 19 kDa C-terminal region of MSP-1,^[10] which have given courage as possible candidates of malaria vaccine development,^[11]. MSP-1 is utilised for the attachment of merozoites to the specific host receptor named Band 3,^[12] and acts as a ligand through which complexes of several extrinsic proteins that bind erythrocytes are formed,^[13] and thus, is essential for erythrocyte parasite invasion and/or growth,^[14,15] and eliciting both Th1 and Th2 immune responses^[11]

Our laboratory had utilised the 19kDa MSP (MSP-1C) of *P. falciparum* in constructing a recombinant BCG (rBCG) vaccine candidate.^[16] This construct was able to elicit innate immune responses in macrophages leading to higher production of pro-inflammatory cytokines, TNF- α and IL-1 β ,^[17, 18] than the parent BCG or MSP-1C alone.^[18] Innate

immune reaction, associated with increased pro-inflammatory cytokines such as IL-1 β , and TNF- α ,^[19, 20] as well as IL-12p40,^[21] is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) by the specialised pattern recognition receptors, PRRs such as toll-like receptor-4,^[22] present on immune cells such as macrophages. The mechanism through which our rBCG construct achieved its immunomodulatory effects had not been characterised. This study tested the hypothesis that TLR-4 mediates the attachment between rBCG and macrophages, a prerequisite to eliciting an innate immune response. The production of Pro-inflammatory cytokines; TNF- α , IL-1 β , and IL-12p40 by peritoneal macrophages in the presence or absence of a TLR-4 inhibitor; TAK-242 was analysed.

Methods

The rBCG strain expressing *P. falciparum* MSP-1C

The recombinant BCG expressing a synthetic gene encoding MSP-1C of *P. falciparum* was earlier cloned in the laboratory through the polymerase chain reaction and transformation into BCG Pasteur by electroporation.^[16]

Preparation of BCG and rBCG cultures

The parent BCG and rBCG (Japan) were cultured on a 7H11 agar (Becton Dickinson, USA) which has been supplemented with oleic acid, albumin, dextrose, and catalase (OADC) (Becton Dickinson, USA), with 15 μ g/mL of kanamycin (Sigma, USA) added to the rBCG were cultured and later transferred to flasks containing 10 mL of 7H9 broth (Becton Dickinson, USA), also supplemented with OADC with 15 μ g/mL of kanamycin for rBCG culture until an optical density (OD) of approximately 0.8 (A600 $\approx$ 0.8) was obtained. The colony forming unit was calculated for both cultures as described by Norazmi and Dale.^[23]

0.1 OD = 4X 10⁶ CFU/mL

Reference drug

The toll-like-4 inhibitor TAK-242 from Cayman Chemical, USA was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1mg/ml stock solution.

Immunisation of Mice

The Universiti Sains Malaysia (USM) Animal Ethics Committee approved all experimental procedures in the study [No: USM/Animal Ethics Approval No.

2016 (104) (801)]. A total of 36 male BALB/c mice aged 4-6 weeks were used for the study. The mice received an intraperitoneal (i.p.) injection of 10⁶ CFU rBCG in 200 μ l phosphate buffered saline (PBS) containing 0.1% Tween 80 (PBS-T80) while control groups of mice were injected with either 10⁶ CFU of the parent BCG strain in 200 μ l PBS-T80 or with 200 μ l PBS-T80 in the presence or absence 0.5 mg/kg of TAK-242 given intraperitoneally,^[24, 25, 26] one hour before each mouse in each group was immunised. Three weeks after the first immunisations two booster immunisations of the same concentrations were given, three weeks apart. The mice were observed for any adverse effects from the immunisations

Peritoneal Macrophage harvest

Three weeks after the last booster, the mice were sacrificed via rapid cervical dislocation, done carefully to avoid blood contamination in the peritoneal cavity. The peritoneal macrophages were then harvested from the mouse as described by Ray and Dittel.^[27] Immediately after sacrificing the mouse, each mouse was sprayed with 70% ethanol and mounted on the Styrofoam block on its back and pinned with pins. An incision was made over the caudal half of the abdomen and skin lining lifted. The mouse was re-sterilised with 70% ethanol and ice-cold sterile PBS (with 3% FBS) gently injected into the caudal half of the peritoneal cavity. The pins were gently removed and the entire body of the mouse was gently shaken and the peritoneum was massaged for 10 seconds to dislodge any attached cells into the PBS solution. A 25 G needle was used to collect the fluid which was deposited in tubes kept on ice.

The suspension collected was spun at 125 x g at 4°C for 8 minutes, the supernatant discarded and the cells resuspended in Dulbecco's Modified Eagle Media (DMEM) and 1 $\times$ 10⁶ total peritoneal cells/mL and transferred to 25 mL flask and cultured for 2 hours at 37°C in a CO₂ incubator. Non-adherent cells were removed by gentle washing three times with warm PBS to get the expected more 90% macrophage,^[28] which was incubated in the 5% CO₂ humidified incubator for another 2 hours at 37°C. The cells were then gently scraped using a sterile plastic scraper and transferred into a 15 mL tube and harvested by centrifuging at 125 x g for 5 minutes.

Pro-inflammatory cytokine assay

For analysis of the effects of TLR-4 on the production of TNF- α , IL-1 β and IL-12p40 by peritoneal macrophages, cell-free culture supernatants harvested were used and the respective concentrations determined by standard sandwich ELISA kits (Bio-Rad). Briefly, a maxisorp 96-well ELISA plate was coated with 100 μ L of capture anti-TNF- α , anti-IL-1 β or anti-IL-12p40 antibody sealed and incubated overnight at 4°C and blocked with PBS containing 0.05% Tween 20 and 10% FBS then 100 μ L of culture supernatants and standard added into the 96-well microtiter plate, sealed and incubated for 2 hours at room temperature followed by 5 washes then 100 μ L of anti-mouse TNF- α , IL-1 β or IL-12p40 was added to each well accordingly, sealed and incubated for 2 hours at room temperature. One hundred μ L of Avidin-HRP solution was added to each well and incubated for 30 minutes at room temperature. Then washed and 100 μ L of substrate solution added and incubated at room temperature for 15 minutes in the dark. Fifty μ L of stop solution was added and the optical density was measured with a microplate reader at 450 nm, subtracting background at 570 nm.

The cytokine concentrations were determined and a standard curve of mouse TNF- α , IL-1 β or IL-12p40 generated. The concentrations of the cytokines for each sample were calculated by regression analysis using the mean absorbance.

Statistical analysis

Statistical analyses were performed using the statistical package of social sciences (SPSS) software version 24. All Data were representative of 3 experiments presented as mean $\pm$ standard error of the mean (SEM). All the data were analysed by one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. The p -value of < 0.05 was considered statistically significant.

Results

TNF- α production by peritoneal macrophages of mice immunised with PBS-T80, BCG or rBCG

To determine whether toll-like receptor-4 affects TNF- α production, the production by macrophages harvested from the peritoneum of mice immunised with the rBCG, the parent BCG or PBS-T80 in the presence or absence of the TLR-4 inhibitor, TAK-242 was measured. As shown in Figure 1, TNF- α was produced in all groups of mice involved in the study with a significantly higher production in the rBCG immunised group (739.483 ± 1.585 pg/mL) then the BCG immunised group (301.582 ± 2.221 pg/mL) while the PBS-T80 immunised group (244.175 ± 2.010 pg/mL) had the least TNF- α production in the absence of TAK-242, $p < 0.05$. The effect of TLR-4 inhibition was demonstrated by the significant inhibition of TNF- α in the presence of TAK-242 in the PBS-T80 immunised groups ($p < 0.05$, and mean difference -118.207), as well as the BCG, immunised groups ($p < 0.001$, and mean difference -147.875) and the rBCG groups ($p < 0.05$, and mean difference -634.498).

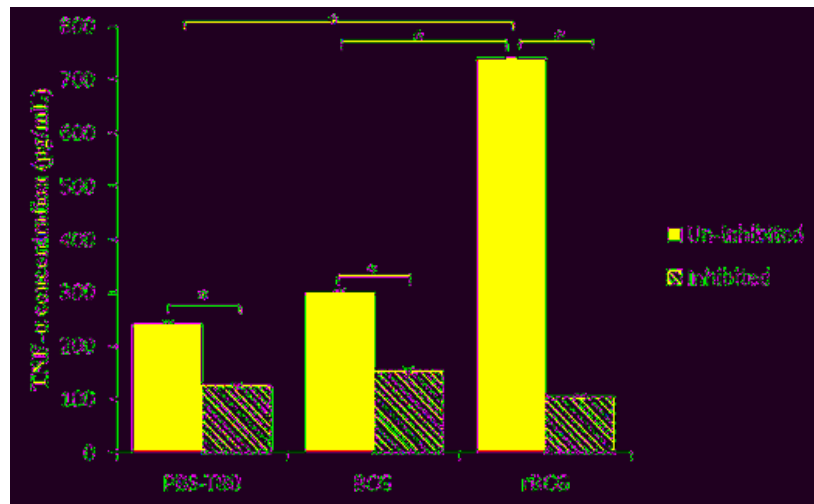


Fig. 1: TNF- α production in peritoneal macrophage of mice immunised with PBS-T80, BCG and rBCG in the presence or absence of TLR-4 inhibitor, TAK-242. Data are presented as the mean concentration of TNF- α $\pm$ SEM for three independent experiments. * $p < 0.05$.

IL-1 β production by peritoneal macrophages of mice immunised with PBS-T80, BCG or rBCG

To determine whether toll-like receptor-4 affects IL-1 β production, the production by macrophages harvested from the peritoneum of mice immunised with the rBCG, the parent BCG or PBS-T80 in the presence or absence of the TLR-4 inhibitor, TAK-242 was measured. As shown in Figure 2, IL-1 β was produced in all groups of mice involved in the study with a significantly higher production in the rBCG immunised group (403.030 ± 2.167 pg/mL) then the

BCG immunised group (246.610 ± 1.897 pg/mL) while the PBS-T80 immunised group (139.940 ± 0.482 pg/mL) had the least IL-1 β production in the absence of TAK-242, $p < 0.05$. The effect of TLR-4 inhibition was demonstrated by the significant inhibition of IL-1 β in the presence of TAK-242 in the PBS-T80 immunised groups ($p < 0.05$, and mean difference -32.260), as well as the BCG, immunised groups ($p < 0.05$, and mean difference -98.741) and the rBCG groups ($p < 0.05$, and mean difference -220.937)

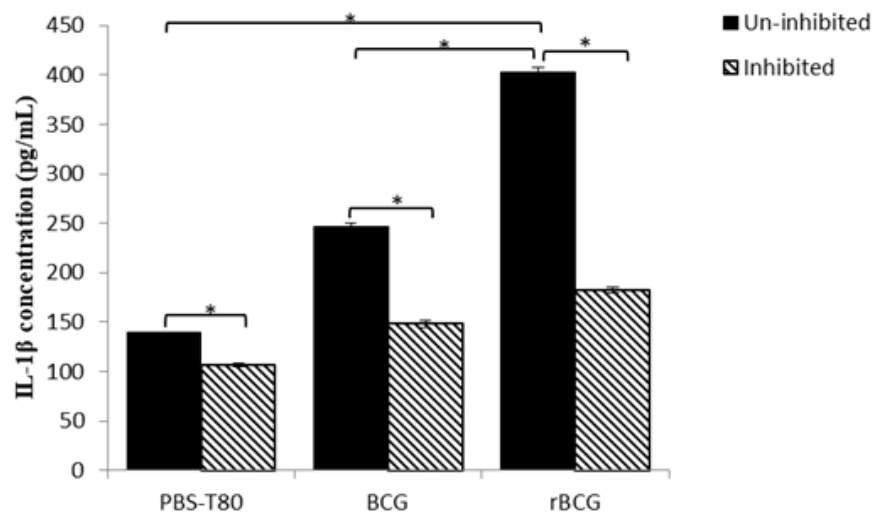


Fig. 2: IL-1 β production in peritoneal macrophage of mice immunised with PBS-T80, BCG and rBCG in the presence or absence of TLR-4 inhibitor, TAK-242. Data are presented as the mean concentration of IL-1 β $\pm$ SEM for three independent experiments. * $p < 0.05$.

IL-12p40 production by peritoneal macrophages of mice immunised with PBS-T80, BCG or rBCG

To determine whether toll-like receptor-4 affects IL-12p40 production, the production by macrophages harvested from the peritoneum of mice immunised with the rBCG, the parent BCG or PBS-T80 in the presence or absence of the TLR-4 inhibitor, TAK-242 was measured. As shown in Figure 3, IL-1 β was produced in all groups of mice involved in the study with a significantly higher production in the rBCG immunised group (456.843 ± 1.736 pg/mL) then the

BCG immunised group (365.974 ± 1.786 pg/mL) while the PBS-T80 immunised group (293.115 ± 0.849 pg/mL) had the least IL-12p40 production in the absence of TAK-242, $p < 0.05$. The effect of TLR-4 inhibition was demonstrated by the significant inhibition of IL-12p40 in the presence of TAK-242 in the PBS-T80 immunised groups ($p < 0.05$, and mean difference -86.625), as well as the BCG, immunised groups ($p < 0.001$, and mean difference -149.133) and the rBCG groups ($p < 0.05$, and mean difference -222.321)

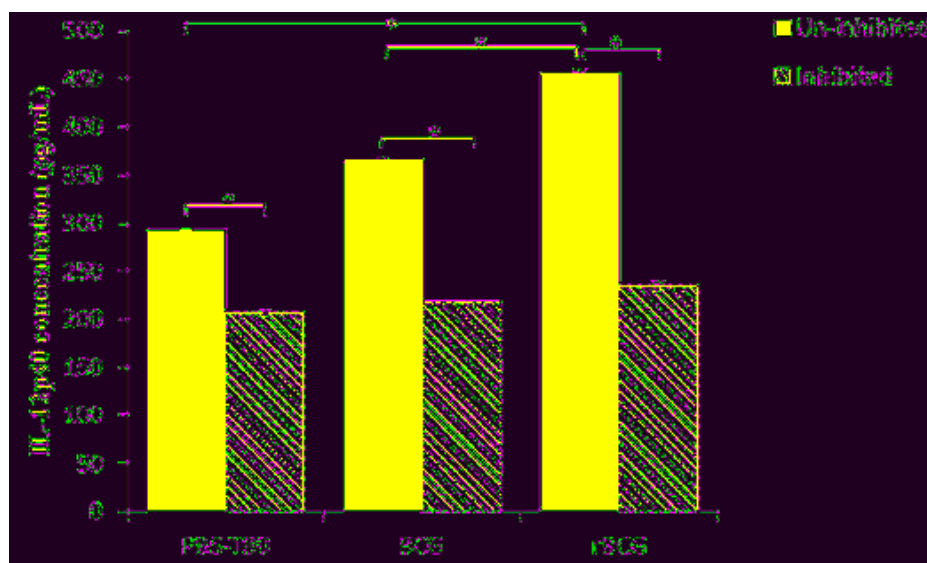


Fig. 3: IL-1p40 production in peritoneal macrophage of mice immunised with PBS-T80, BCG and rBCG in the presence or absence of TLR-4 inhibitor, TAK-242. Data are presented as the mean concentration of IL-12p40 $\pm$ SEM for three independent experiments. $p < 0.05$.

Discussions

Macrophages are a class of immune cells which serve as the first line of defence leading to inflammation,^[29] coordination of innate and adaptive immune responses, and tissue repair,^[30, 31] through stimulation of the TLRs by ligands which lead to the upregulation of various pro-inflammatory cytokines,^[32, 33] such as TNF- α , IL-1 and IL-12 (34). In the case of malaria, TLRs interactions with malarial surface molecules lead to the release of a number of IL-1, IL-6, IL-12, and TNF- α .^[35] Recognition and presentation of the components of malaria parasite have been achieved via TLR-4 among others,^[36] and is specific for purified GPI of the *P. falciparum*,^[37] via the MyD88-dependent pathway.^[38]

This study looked into the role of TLR-4 in the activation of peritoneal macrophages of immunised mice and found a robust production of pro-inflammatory cytokines; IL-1 β , TNF- α , and IL-12p40 in all groups of mice immunised with PBS-T80, BCG or rBCG. The results obtained showed significantly higher production of the cytokines in the group immunised with rBCG than in those immunised with BCG or PBS-T80. A similar picture of increased cytokines release was found in all clinical cases of malaria infection. ^[39] This study found significant inhibition of all the pro-inflammatory in the presence of the TLR-4 inhibitor, TAK-242 signifying its role in macrophage activation and cytokine production. In the same vein,

a study on the role of the TLR-4-mediated pathway in eliciting inflammation in acute lung injury found that *Sanghuangporus sanghuang* exhibited significant inhibition of LPS-induced increased secretion of both TNF- α , and IL-1 β , in broncho-alveolar lavage fluid.^[40] Another study found that oral and intraperitoneal administration of lignin-rich enzyme lignin (LREL), a cellulase-treated lignin-carbohydrate derived from plants stimulated the innate immune system leading to increased IL-12p40 production.^[41] While another study went further to discover that LREL-induced IL-12p40 and TNF- α production by dendritic cells (DCs) of TLR-4/- mice was significantly reduced.^[42] Injection of lipopolysaccharide (LPS) enhanced arthritis via TLR-4-mediated IL-12 production which in turn stimulates IFN- γ and IL-1 β production by joint macrophages was also significantly reduced in TLR4-/- mice.^[43]

When the production of IL-1 β was analysed we found significantly higher production in the group immunised with the rBCG than those immunised with PBS-T80 and BCG. These findings confirmed the immunomodulatory effects of the construct found in an earlier in vitro study, which elicited higher IL-1 β production in J77.4 macrophage cell lines treated with the construct.^[17, 18] Interestingly, when the role of TLR-4 in eliciting the production of IL-1 β was evaluated through its inhibition by TAK-242, the results obtained showed a significant inhibition of the cytokine production. These results were similar to what obtained in a study where the inhibition of TLR-4 with TAK-242 led to a significant inhibition of IL-1 β production in microglia.^[25] LPS-induced IL-1 β production in monocyte/ macrophage cell line termed THP-1 cells and human white blood cells was also inhibited by a synthetic trehalose diester, vizantin, a TLR-4 inhibitor,^[44] while TLR-4-/- mice recipients of orthotopic small intestine grafts also had inhibited IL-1 β .^[45]

This study also found a significantly higher TNF- α production in the group immunised with the rBCG compared to the control groups, similar to what obtained previously with the construct in J77.4

macrophage cell lines.^[17, 18] Again, TLR-4 inhibition with TAK-242 led to a significant inhibition in the production of TNF- α in all mice groups similar to what others found earlier.^[25, 45] Additionally, a study on allografts showed that inhibition of TLR-4 via TAK-242 treatment significantly reduced the production of TNF- α ,^[46, 47, 48] as was LPS-induced TNF- α production in prefrontal cortex (PFC) of pleiotropin transgenic mice (PTN-Tg mice).^[49] TLR-4-/-macrophages,^[50] and small interfering RNA for TLR-4,^[44] showed a significant reduction in TNF- α production.

The production of IL-12p40 in the peritoneal macrophages of the various groups of immunised mice was also in favour of the rBCG. This was similar to what obtains in cerebral malaria,^[51] uncomplicated *P. vivax*,^[52] and tuberculosis,^[53] infection which showed higher production of IL-12p40 among others. Expectedly, TLR-4 inhibition via TAK-242 treatment led to a significant inhibition of the otherwise robust IL-12p40 production signifying the role of toll-like receptor-4 in the activation of the macrophages similar to its effects on allografts where it exhibited significantly reduced expression of IL-12.^[49]

Conclusion

There is a robust production of the pro-inflammatory cytokines IL-1, TNF- and IL-12p40 in the innate immune response to our BCG-based malaria vaccine expressing the MSP-1 of *P. falciparum*. This could be due to the coupling of TLR-4 with its ligand, the MSP-1C of *P. falciparum* which led to enhanced bonding between macrophages and the vaccine. The vaccine candidate has been shown to activate the MyD88 pathway.^[54]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Author Contributions

M.A.A. performed the research and analysed the data. R.S. designed the experiments and analysed the data. All authors drafted and approved the final manuscript.

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Effect of Aqueous Leaves Extract of *Cassia Occidentalis* on the Histology of Liver of Adult Wistar Rats

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Abstract

Cassia occidentalis is a medicinal plant traditionally in the north-western part of Nigeria for the treatment of typhoid and malaria. The liver has about 500 functions; detoxification is one of the major functions of the liver. The study aimed at determining the effects of aqueous extract of *cassia occidentalis* leaf on the histomorphology of the liver of adult Wistar rats. Sixteen rats were grouped into group A, B, C and D, each group contains four rats. Group A administered distilled water served as control group and 100mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight of aqueous extract of *Cassia occidentalis* leaf administered to group B, C, and D respectively for 14 days consecutively. On the fifteenth day, the rats were sacrificed and the liver was carefully harvested and fixed in 10% formal saline for histological examinations. The extent of hepatotoxicity was evaluated by histomorphology of the liver. Extract of *Cassia occidentalis* significantly affected the histomorphology of the liver of the Wistar rats compared to the control group. The effects were dose-dependent, which causes the degeneration and necrosis of hepatocytes and degeneration and occlusion of central vein of the liver. It was concluded from the study that *Cassia occidentalis* was hepatotoxic as it affects the histomorphology of the liver.

Keywords: *Cassia Occidentalis*, Liver, Histomorphology, Wistar Rat

Introduction

Plants have been used as traditional medicine and pharmacopoeial drugs from ancient times. Most of the world's population depend on plants due to their medicinal value; ^[1,2] medical plants have been used for treatment of illness since ancient period. ^[3] Numerous plant-derived therapeutic agents for modern medicine have been provided by medicinal plants. ^[4,5] Most of the plants exhibit a variety of phytopharmaceuticals, which has important applications in the fields of agriculture, human and veterinary medicine. This plays a major role in developing novel drugs for the treatment and prevention of diseases.^[5]

Therefore, it is very important to have sufficient knowledge regarding herbs not only because of their widespread uses but also because they have the potentials to cause toxic reactions or interact with other drugs.^[6] Although in traditional medicine *Cassia* species have been well known for their laxative and purgative properties and for the treatment of skin diseases.^[7] *Cassia occidentalis* Linn has been used as a folklore medicine for

hepatotoxicity treatment.^[8] There is now an increasing body of scientific evidence demonstrating that the plants possess many other beneficial properties.

The liver is the largest and heaviest internal organ, it plays a central role in the transforming and clearing of chemicals in the body hence it is susceptible to toxicity from these chemical agents. Interest in the evaluation of herbal remedies on vital organs has increased due to certain drawbacks in the application of these remedies mainly an increase in the incidence of liver diseases.^[9] Substantial progress has been made in the understanding of interactions between herbal drugs and the liver.

However, knowledge of the potentials and risks is still limited and efforts to elucidate them are ongoing. Studies have identified certain herbal remedies as hepatotoxins. People from the northwestern part of Nigeria are using *Cassia occidentalis* randomly as herbal medicine to cure mainly malaria and typhoid fever without knowing

the consequence on organs like liver. Hence, the justification for the research on its effects on the cytoarchitecture of the liver. Moreover, there is a paucity of data in the Northwestern region of Nigeria, regarding the effect of *Cassia occidentalis* especially on the histomorphology of the liver, henceforth this research provides additional information about the effect of this herb (*cassia occidentalis*). This study may provide an insight into the limited dose of the herbal drug for safe use. The aim of the study was to determine the effect of aqueous leaves extract of *cassia occidentalis* on the liver histology of adult Wistar rats.

Materials and Methods Animal Procurement

Albino Wistar rats (both male and female) weighing 100-110g were obtained from the Department of Human Physiology, Bayero University Kano and acclimatize for two weeks (14 days) in the Department of Anatomy, Bayero University Kano. Animals were housed in colony cages at ambient temperature and relative humidity. The animals have free access to standard panellised grower feed (VITAL) and drinking water ad libitum. All authors hereby declare that the principle of laboratory animal care According to NIH Guidelines^[10] for the care and use of laboratory animals and ethical guidelines for investigation of experimental animal pain in conscious animals were observed during experimentation.

Plant Material

The leaves of *Cassia occidentalis* were collected fresh from a forest at Shanono Local Government in Kano State. The leaves were identified and authenticated at Department of Plant Science, Bayero University Kano with identification number BUK/HAN/0073. *Cassia occidentalis* leaves were dried under the shade and ground into powder. Textraction was performed by soaking 400g of the crude powder of the leaves of *Cassia occidentalis* in two litres of distilled water for about 28hours. It was filtered using three layers of cheesecloth before using Whatmann filter paper to obtain a clear debris-free extract. The filtrate was then evaporated to dryness in a water bath at 60°C. The solvent-free extract was dissolved in distilled water and administered to rats. The volume to be administered to animals was calculated according to the method of Muhammad *et al.*^[11]

Weighing and Grouping of Animals

The rats were weighed and grouped into four (4) groups of four rats in each separate cage. The extract was administered as follows.

GROUP A: administered distilled water (which served as the control group)

GROUP B: Administered with 100mg/kg of *Cassia occidentalis* leaves extract

GROUP C: Administered with 200mg/kg of *Cassia occidentalis* leaves extract

GROUP D: Administered with 300mg/kg of *Cassia occidentalis* leaves extract.

The treatment was through the oral route and lasted for two weeks (14days).

Collection of Tissue

The rats were anaesthetised with chloroform and were sacrificed humanely. The liver tissue was located and harvested via an abdominal incision.

Tissue Processing

There are four (4) main stages in tissue processing namely; Fixation, Dehydration, Clearing and Infiltration, tissue Sectioning and Slide Mounting using routine histological staining method Haematoxylin and Eosin

Photomicrographs

The tissue sections were examined under a light microscope (Olympus) with a digital camera (Amscope) attached. Digital photomicrographs of the tissue sections were taken at various magnifications (40-100).

Results

Plate IA is a photomicrograph of a liver section of the control group seen in lower magnification (×40), which was administered distilled water and used as a control group. The photomicrograph indicated the normal histology of a liver presenting radiating hepatocytes, (HC) around the central vein (CV). The sinusoids(S) were normal and still maintained the hepatic cytoarchitecture. Plate IB at higher magnification (×100), showed the prominence of the normal hepatocytes (H) interspersed by the normal sinusoid(S).

The histopathological findings obtained in this study showed that aqueous extract of *Cassia occidentalis*

leaf was hepatotoxic as seen in the liver sections of Wistar rats characterised as follows:

Plate IIA, lower magnification ($\times 40$) is a section of the liver of a Wistar rat in group B administered 100 mg/kg body weight of aqueous extract of *Cassia occidentalis* leaf showed the occlusion of central vein and also haemorrhage in the central vein, distortion of the hepatic cyto-architecture. While the Plate IIB, which is in higher magnification ($\times 100$), showed the prominence occlusion of central vein and some element of hepatic degeneration.

Plate IIIA, lower magnification, is the group-administered 200 mg/kg body weight of the extract,

showed the degeneration and occlusion of central vein. While the Plate IIIB, in higher magnification ($\times 100$), showed the degeneration of central vein, karyolysis of hepatocytes and disorientation of hepatic sinusoid.

Plate IVA: in lower magnification ($\times 40$), is the group administered 300 mg/kg body weight of the extract, showed the disorientation of hepatic sinusoid, degeneration of central vein and hepatocytes. Plate IVB is the higher magnification ($\times 100$), showed the degeneration of central vein and hepatocytes, necrosis of hepatocytes and occlusion of the central vein.

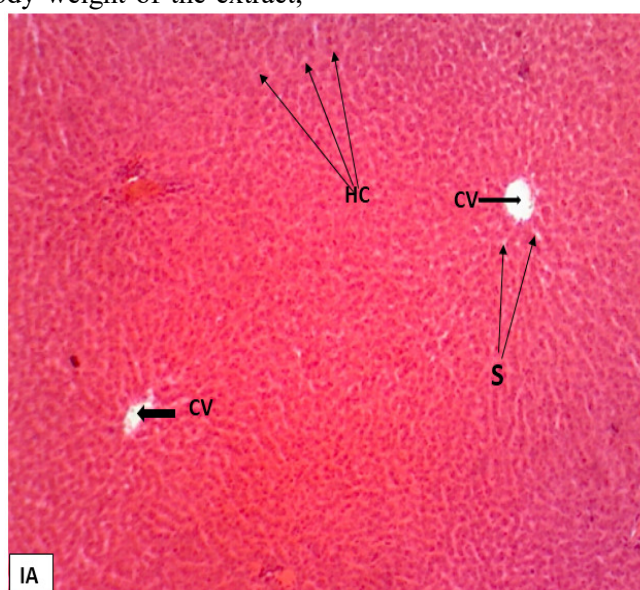


Plate IA: Control; Normal Rat Liver showing CV: central vein, HC: hepatocytes, S: sinusoid (H&E $\times 40$)

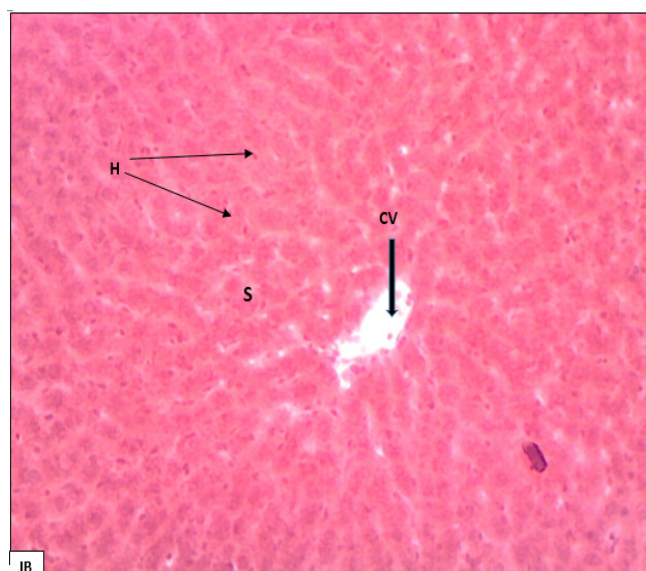


Plate IB: Control; Normal Rat Liver showing CV: central vein, HC: hepatocytes, S:sinusoid (H&E×100)

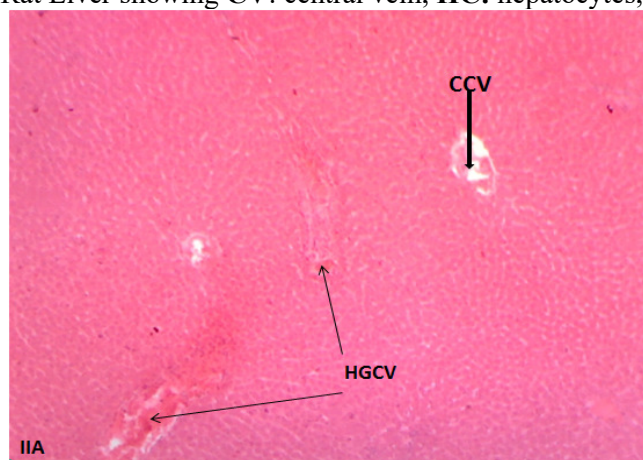


Plate IIA: Rat Liver administered 100mg/kg body weight of *C. occidentalis* aqueous extract showing haemorrhage in the central vein(HCV), Congestion or occlusion of the central vein (CCV) and distortion of hepatic cytoarchitecture(H&E ×40)

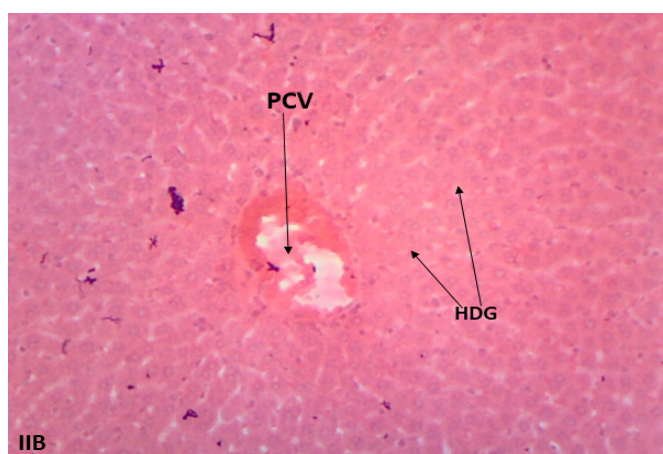


Plate IIB: Rat Liver administered 100mg/kg bodyweight of *C.occidentalis* aqueous extract showing the prominence Occlusion of central vein (PCV), Hepatic degeneration (HDG). (H&E ×100)



Plate IIIA: Rat Liver administered 200mg/kg body weight of *C.occidentalis* aqueous extract showing occlusion of central vein (OCCV), degeneration of central vein(DCV).(H&E ×40)

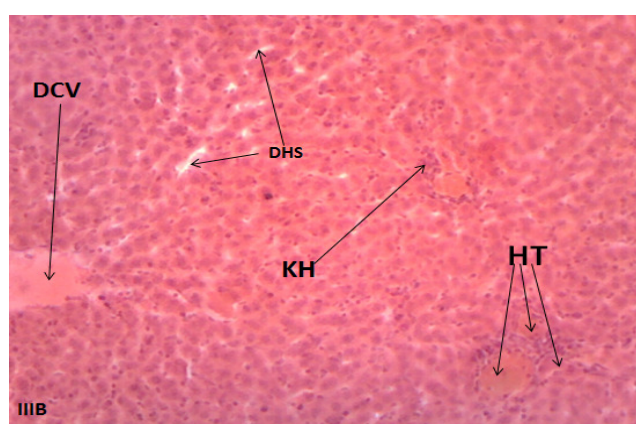


Plate IIIB: Rat Liver administered 200mg/kg bodyweight of *C. occidentalis* aqueous extract showing disorientation of hepatic sinusoid(DHS), degeneration of central vein (DCV) karyolysis(KH), hepatic triad(HT).(H&E ×100)

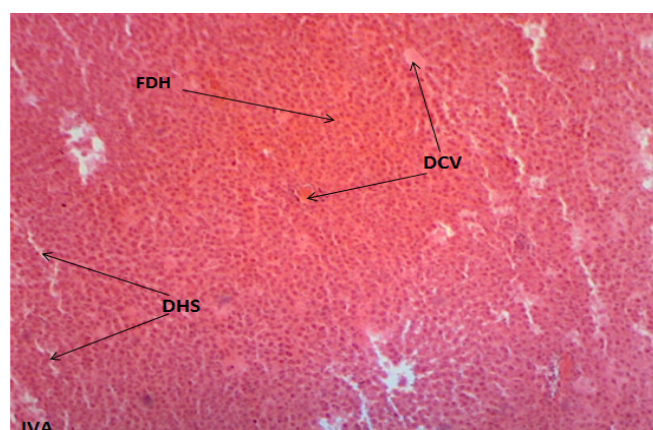


Plate IVA: Rat Liver administered 300mg/kg bodyweight of *C. occidentalis* aqueous extract showing Disorientation of hepatic sinusoid (DHS), Degeneration of central vein (DCV), Further degeneration of hepatocytes(FDH). (H&E ×40).

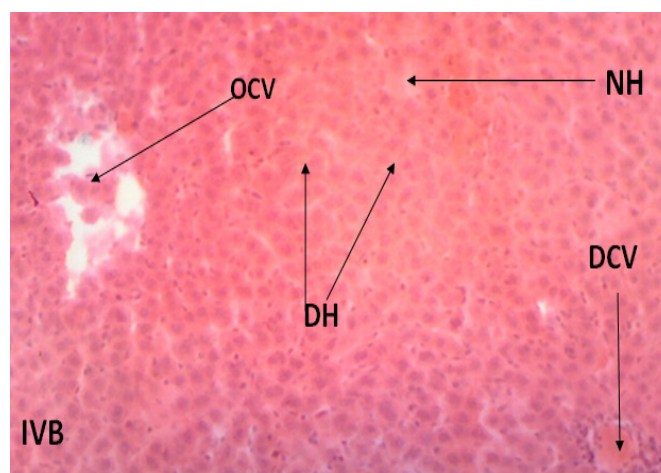


Plate IVB: Rat Liver administered 300mg/kg body weight of *C.occidentalis* aqueous extract showing Occlusion of central vein(OCV), Necrosis of the hepatocyte (NH), Degeneration of central vein (DCV), Degeneration of hepatocytes(DH). (H&E ×100).

Discussion

The result of the study demonstrated that *Cassia occidentalis* is hepatotoxic in the rats model; this is in line with earlier or acute observation on the effect of aqueous extract of *C. occidentalis* leaf on the liver of Wistar rats which showed that it was hepatotoxic as it caused the degeneration, karyolysis and necrosis of hepatocytes, degeneration and occlusion of central vein, haemorrhage in the central vein and also disorientation of sinusoids. The damage to the liver lead to release of toxic necrotic substances into the blood; this will further lead to the intoxication of central nervous system and possible hepatic coma. The histopathological changes were dose-dependent because the rats treated with 200 mg/kg and 300 mg/kg body weight were mostly affected while those treated with 100 mg/kg body weight showed little effect. This results were similar to that of Chris-Ozoko *et al.* ^[12] who reported that the aqueous extract of *C. occidentalis* leaves causes degeneration and necrosis of hepatocytes. Apart from the leaves extract, the aqueous root extract of *C. occidentalis* caused the degeneration and necrosis of hepatocytes. ^[13] As the aqueous extract of *C. occidentalis* has an effect on the histomorphology of liver of Wistar rats, it may likewise have similar effects on other organs such as kidney by causing degeneration and inflammation of glomerulus and inflammation of renal tubules.

Conclusion

The findings of this study demonstrated that the aqueous extract of leaves of *Cassia occidentalis* are

hepatotoxic as it affects the histomorphology of the liver at different concentrations used. This requires that caution be maintained in its use as a herbal drug.

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The Effect of Urbanisation on Maternal and Neonatal Anthropometric Parameters among the Mother-Child Pair in Kano, Nigeria

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Abstract

It has been observed that the mother's residence (either urban or rural) is strongly associated with low neonatal anthropometric values, such that mothers residing in rural areas are discovered to be four times more likely to have low birth weight (LBW) babies compared to those who live in the urban areas. Separate studies have emphasized that significant associated risk factors of anthropometric parameters after delivery such as neonatal birth weight, vary according to geographical location. Also, maternal anthropometric parameters such as maternal weight, height, and the maternal arm circumference have been used to predict poorer anthropometric parameters immediately after delivery. In the present study, an attempt was made to determine how urbanisation (area of residence) affects both the maternal and neonatal anthropometric parameters among the mother-child pair in Kano State, Nigeria. A sample of 1203 subjects (mother-child pair) was selected randomly who gave birth at the six selected hospitals (three hospitals from the metropolitan and three from each of the senatorial zone) within Kano State. It was observed that in all the parameters considered, urban parameters were higher (0.001) than those obtained among the rural populace.

Keywords: Maternal Anthropometric Parameters, Mother-Child Pair, Neonatal Anthropometric Parameters, Urbanisation.

Introduction

It has been shown that the mother's residence is strongly associated with low neonatal anthropometric values, as mothers residing in rural areas are discovered to be four times more likely to have low birth weight (LBW) babies compared to those who live in urban areas.^[1] Similarly, it is shown that mothers in rural areas tend to give birth to LBW babies than women who live in urban areas.^[2] It is revealed that nearly all of the newborns who die immediately after their delivery are of LBW and are mostly in rural communities where weighing scales are not readily available.^[3] Reports by^[4,5,6,7] showed that more than half of these LBW babies were deliveries from rural families at home, and the babies die shortly after birth. Separate studies conducted by^[8,9] have emphasized that significant associated risk factors for the birth weight of a newborn, vary according to geographical location and study population.

Maternal anthropometric parameters such as maternal weight, height, and maternal arm circumference have been used to predict a poorer birth outcome.^[10] An earlier report shows that a strong correlation exists between maternal height and birth weight.^[11] Similarly, it was reported that there is an existence of correlation between birth weight and maternal anthropometric parameters that is age of the mother, maternal parity and gestational age.^[12] Several studies have emphasised that short maternal height is associated with reduced foetal growth and low birth weight.^[13,14,15,16] Though studies on anthropometric parameters on neonates in Kano State had been undertaken in the past.^[17,18] to the best of our knowledge, there has not been any study conducted on several neonatal anthropometric parameters at a time among the neonates of Kano State.

Materials and Method Materials

The materials used for the study include Digital Baby Weighing Scale (EBSA-20, Kologn Industrial Limited, China), Compact Bowl Digital Scale (LB-3000, American Weigh Scales Inc., China), Stadiometer (ZT – 160, Seca, USA), Sphygmomanometer [Accoson, A.C. Cossor & Sons (surgical) Ltd., England], Plastic non elastic measuring tape (Butterfly, Chinese Butterfly Company, People's Republic of China), Plastic meter rule (TR15, Bestlink rule, China), Skin pencil and Disposable hand gloves.

Study Design

This is a prospective, cross-sectional, hospital-based study conducted among maternal-neonatal (mother-child) pairs who gave birth at the six selected hospitals within Kano State from November 2017 to May 2018. The selected hospitals within the metropolis (urban area) include Murtala Muhammad hospital, Muhammad Abdullahi Wase Specialist Hospital, and Jakara Maternity Hospital. Among the selected hospitals from the rural areas, one hospital is selected from each of the senatorial zones that include Bichi General Hospital, Kura General Hospital, and Gaya General Hospital. The selection of the hospitals was done using simple random sampling (SRS) by listing the hospitals in each of the senatorial zones which formed the sampling frame. A random number was chosen using the random number table, and the six hospitals were selected accordingly. All measurements were recorded on the designed proforma. The study subjects were recruited from the parturition mothers brought to the hospitals. The subjects were briefed on the study and its likely outcomes as it appeared on the consent form. Recruitment for the study was only made upon signing the written consent form (by the mothers). The inclusion criteria include signing of a written consent form (by the mother), term (of 37 completed weeks) singleton babies that were delivered within 24 hours (1 day) not having any physical deformity. The selection of participants was done from the successive admissions in the delivery units of the selected hospitals. Therefore, it was only those who fulfilled the criteria below that were considered eligible to get involved in the study.

Method

Ethical clearance was obtained from the Ethical Committee of Kano State Ministry of Health. The

data were collected in each of the selected hospitals, two research assistants (which are regular, and full-time midwives) working in the delivery room, were trained on the standard procedures of measuring and recording the selected parameters on a proforma.

Maternal and Neonatal Anthropometric Measurements

All the maternal and neonatal parameters were taken using standard techniques,^[19, 20] and by using the left side of the body.^[21, 22] The measurements were taken within 24 hours of delivery using the same (identical) equipment provided to all the sites. The measurement procedures were standardized based on the World Health Organization's (WHO) recommendations to ensure maximum validity.^[23] During the standardization sessions, the intra-observer and inter-observer error of measurement values for the measured neonatal and maternal anthropometric parameters were done by the same or different person as appropriate (i.e. intra & inter-observer error). This is done by obtaining a sample of 35 at random; the results obtained were tested using Pearson's correlation (r). Values greater than 70% were accepted and recorded, while values less than 70% were repeated twice and even thrice where appropriate.

The Maternal Anthropometry

Measurements were carried out following the method of Talat *et al*^[24] as

Maternal Height (MH)

The maternal height was taken using the ZT-Seca stadiometer with minimal clothing. The pregnant mother was asked to maintain an upright and erect posture with her feet together (Frankfort position). Height was measured in cm as a straight distance between the head and the barefoot. This was done by lowering the horizontal headpiece that is attached to the stadiometer onto the woman's head. Reading was recorded to the nearest cm.

Maternal Arm Circumference (MAC)

The maternal arm circumference (MAC) was measured using a non-elastic plastic measuring tape as a distance midway between the tip of the shoulder joint (acromium) and the tip of the elbow joint (olecranon process). The measurement was done by bending the examination hand at the elbow joint. With the arm hanging straight down, the tape

was wrapped up around the arm at the midpoint mark. Measurement was recorded to the nearest 1 cm in the proforma.

Symphysis-Fundal Height (SFH)

The symphysio-fundal height was measured using a non-elastic plastic measuring tape as the distance between the symphysis pubis and the highest point of the uterine fundus, with the mother in the supine position. The measurement was made from the upper edge of the symphysis pubis following the curvature of the abdomen with the tape; the upper hand was placed firmly against the top of the fundus with the measuring tape passing between the index and middle fingers. Readings were taken from the perpendicular intersection of the tape with the fingers.

Haemoglobin (Hb g/dl) Estimation

The haemoglobin testing was made with R. TERRY haemoglobin scale (Rub. Terry ind. & Co. Ltd) using Tallquist absorbent paper. It requires a small (a drop) sample of easily obtained blood. Before the blood was taken, the middle finger was wiped with a swab dumb in a methylated spirit and allowed to air-dry. Then the palm side of the end of the middle finger was pricked with a sterile, non-reusable, self-retractable lancet and a drop of blood was collected using a Tallquist absorbent paper. The Tallquist absorbent paper was slowly brought in contact with the drop of blood. Next, the paper was compared with the haemoglobin scale by holding it against the opening on the haemoglobin scale. Each hole has a different colour which can be compared with the colour of the blood on the absorbent paper. The haemoglobin scale can read the haemoglobin level of 4.7 g to 15.6 g (equivalent to Hb or PCV of 14.1 to 46.8% respectively). All the readings obtained were converted and recorded in g/dl.

The Neonatal Anthropometry

The neonatal anthropometric parameters were measured within 24 hours of delivery ^[25] as:-

Birth Weight (BWT)

The BWT was taken in g using a digital weighing scale with a capacity of up to 20,000 g and a resolution of 0.00 g. The weighing scale was cleansed with a sterilized drape, and then the baby was placed on the weighing scale naked. Reading was recorded to the nearest g.

Birth Length (BL)

The BL was measured as the projective distance between the highest point on the head (vertex) and the most posterior projecting point of the heel in the supine position. The measurement was carried out by two healthcare providers using a non-elastic measuring tape. The neonate was kept in the supine position (face-up). The first healthcare provider correctly positioned the neonatal head against a vertical flat surface and gently holds the head in its position and the area was marked. The second healthcare provider gently presses the neonate's knees to straighten the legs; the heel position was marked. The distance between the two marked surfaces was measured and recorded on the proforma to the nearest 0.1 cm.

Foot Length (FL)

The FL was measured in cm as a straight distance between the most posterior projecting point of the heel and anterior projecting point (the end of 1st or 2nd toe) using a meter rule from the plantar surface of the sole of the foot.

Foot Breadth (FB)

The FB was measured in cm (at the widest point of the sole) as the distance between the first to the fifth metatarso-phalangeal joint of the foot, using a meter rule from the plantar view of the sole of the foot.

Thigh Circumference (TC)

Thigh circumference was measured with the measuring tape placed around the mid-thigh at the midpoint between the knee and the hip joint. The measuring tape was placed around the mid-thigh. The tape was positioned perpendicular to the long axis of the thigh with the zero ends of the tape held below the measurement value. The tape rests firmly on the skin without compressing the skin. The thigh circumference was measured to the nearest 0.1 cm.

Hand Length (HL)

The HL was measured in cm from the mid-point of the distal wrist crease to the tip of the middle finger using a plastic meter rule from the palmer surface of the left hand.

Hand Breadth (HB)

The HB was measured in cm as a distance from the base of second to fifth proximal phalanx (metacarpo-

phalangeal joint) of the hand using a plastic meter rule from the palmer surface of the hand.

Neonatal Arm Circumference (NAC)

The neonatal arm circumference was measured in cm as the circumference between the midpoint of the tip of the acromion process and the olecranon process of the ulna, with the newborn in dorsal position (decubitus) and with arm lying laterally to the trunk. The measuring tape was placed around the upper arm at the marked point perpendicular to the long axis of the arm. The tape rests on the skin surface but not pulled tight enough to compress the skin. The arm circumference was recorded to the nearest 0.1 cm.

Chest Circumference (CC)

The CC was measured with the tape placed along with the point of nipples, round the back. Measurements were recorded to the nearest 0.1 cm.

Head Circumference (HC)

The HC was obtained by placing tape along the largest occipitofrontal diameter. The tape was placed across the frontal bones just above the eyebrows (glabella), around the head above the ears on each side, and over the occipital prominence at the back of the head. The tape was held snugly around the head. The tape was moved up and down over the back of the head to locate the maximal circumference of the head. The tape was kept perpendicular to the long axis of the face and was pulled firmly to compress the hair and underlying soft tissues. The measurement was recorded to the nearest 0.1 cm.

Statistical Analyses

Data were expressed as Mean $\pm$ Standard Deviation (SD). Tables were used in expressing the data, while an independent sample *t*-test was used to determine the differences between the variables. Data analyses were carried out using IBM SPSS version 25.0 statistical software. $P < 0.05$ was considered statistically significant.

Results and Discussion

Maternal and neonatal anthropometric parameters are important and easily used parameters in evaluating or assessing pregnant mothers, their fetuses in-utero and their newborn babies. However, the importance of these parameters in the

identification of pregnant mothers and their unborn babies and the effect of urbanisation has so far received less attention. The mean maternal height (MH), maternal arm circumference (MAC), symphysio-fundal height (SFH) and Haemoglobin (Hb) concentration in the current study were 160.22 ± 5.82 , 26.60 ± 3.15 , 37.46 ± 0.93 and 12.18 ± 1.40 respectively. When urbanisation was considered, a significantly higher mean SFH (37.52 cm) was observed in mothers domiciled in the urban areas of Kano State ($P < 0.005$) as compared to those in the rural areas. In addition, the BW of neonates observed in the urban centres were significantly ($P = 1.27 \times 10^{-16}$) higher than those observed from the rural areas. These disparities between the urban and rural environments may be due the non availability of some healthcare indices.

This includes determinants such as social, economic and physical barriers to good healthcare such as good road networks to the available healthcare facilities and efficient antenatal care delivery. In the evaluation of 200 pregnant mothers at Jinnah Postgraduate Medical Centre, Karachi, the mean MH was recorded as 152.9 cm and that for the MAC was 28.8 cm. These values are slightly different from that obtained in this study. Racial differences between the study populations may underlie the differences in the maternal parameters observed in this study when compared to the literature. Moreover, the haemoglobin (Hb) level in the current study was observed to be 12.18 g/dl. The values of Hb in the samples of study participants recruited from rural centres in the current study is recorded to have a mean value of 11.45g/dl, which is significantly ($P = 5.42 \times 10^{-57}$) different from the overall mean values for Hb observed for study participants from urban areas (12.69 g/dl). Nevertheless, haemoglobin (Hb) level of less than 11 g/dl in the second trimester of gestation was reported as anaemia in pregnancy.^[26] In addition, haemodilution may be markedly increased in early pregnancy.

Thus, anaemia in pregnancy may include Hb concentration of less than 10 g/dl.^[27, 28] The maternal Hb concentration among rural dwellers is discovered to be lower and these categories of women are more prone to developing anaemia in pregnancy, probably due to non availability of adequate healthcare facilities in the rural settings.

When the evaluation of the neonatal birth weight was based on the urban-rural location of the hospital from which the data collection was done, the mean birth weights were 3.2 kg and 2.9 kg for the urban and rural areas respectively. The variation in the mean BW value in which location is considered and the one in which location is not considered is significant as $P\text{-value} = 1.27 \times 10^{-16}$ (i.e. $P < 0.0001$). This shows that the level of urbanisation significantly affects BW. These findings are similar to those obtained by Michael *et al.* [29] which shows that mothers in rural areas tend to give birth to LBW babies than those mothers who live in urban areas. When location (urban or rural residence) was further considered in this study, it is evident that there is a significant effect of urbanisation on the other measured neonatal anthropometric parameters such as birth length ($P = 5.14 \times 10^{-5}$), foot length ($P = 0.024$), foot breadth ($P = 3.22 \times 10^{-4}$), chest circumference ($P = 0.015$) and head circumference

($P = 0.009$). However, no significant effect of urbanisation is seen on thigh circumference, hand length, handbreadth and neonatal arm circumference.

Furthermore, Tables 3 and 4 indicate the sex-specific effect of urbanisation on anthropometric parameters among the neonates in Kano State. In males, similar patterns were observed as in the general study population except that urbanisation has no significant effect on chest and head circumferences (Table 3). For female neonates, no significant effects of urbanisation on foot length and head circumference were recorded. However, birth weight, birth length and foot breadth appear to be affected by urbanisation in a similar pattern with male neonates. Moreover, chest circumference is discovered to be of no significant effect on ($P = 0.046$) the neonates from the rural area (Table 4).

Table 1: The effects of urbanisation on maternal anthropometric parameters

Variables	Domicile	N	Mean $\pm$ SD	t-value	P-value
MH (cm)	Rural	486	159.84 $\pm$ 5.78	-1.88	0.0598
	Urban	717	160.48 $\pm$ 5.83		
	All	1203	160.22 $\pm$ 5.82		
MAC (cm)	Rural	486	26.47 $\pm$ 3.05	-1.11	0.2679
	Urban	717	26.68 $\pm$ 3.21		
	All	1203	26.60 $\pm$ 3.15		
SFH (cm)	Rural	486	37.37 $\pm$ 0.86	-2.81	0.0050
	Urban	717	37.52 $\pm$ 0.96		
	All	1203	37.46 $\pm$ 0.93		
Hb (g/dl)	Rural	486	11.45 $\pm$ 1.03	-16.79	5.42×10^{-57}
	Urban	717	12.69 $\pm$ 1.39		
	All	1203	12.18 $\pm$ 1.40		

MH; Maternal Height, MAC; Mid-Arm Circumference, SFH; Symphysio-Fundal Height, Hb; Haemoglobin, SD; Standard Deviation

Table 2: The effect of urbanisation on neonatal anthropometric parameters

Variables	Domicile	N	Mean $\pm$ SD	t-value	P-value
BW (g)	Rural	486	2938.68 $\pm$ 429.80	-8.40	1.27×10^{-16}
	Urban	717	3174.62 $\pm$ 508.28		
	All	1203			
BL (cm)	Rural	486	49.48 $\pm$ 3.26	4.06	5.14×10^{-5}
	Urban	717	48.69 $\pm$ 3.37		
	All	1203			
FL (cm)	Rural	486	8.21 $\pm$ 0.63	2.25	0.024
	Urban	717	8.12 $\pm$ 0.66		
	All	1203			
FB (cm)	Rural	486	3.12 $\pm$ 0.36	-3.61	3.22×10^{-4}
	Urban	717	3.20 $\pm$ 0.41		
	All	1203			
TC (cm)	Rural	486	14.76 $\pm$ 2.13	-0.23	0.818
	Urban	717	14.79 $\pm$ 2.03		
	All	1203			
HL (cm)	Rural	486	6.82 $\pm$ 0.47	1.54	0.123
	Urban	717	6.77 $\pm$ 0.54		
	All	1203			
HB (cm)	Rural	486	3.42 $\pm$ 0.31	-0.70	0.482
	Urban	717	3.44 $\pm$ 0.34		
	All	1203			
NAC(cm)	Rural	486	11.06 $\pm$ 1.37	1.14	0.253
	Urban	717	10.97 $\pm$ 1.35		
	All	1203			
CC (cm)	Rural	486	29.75 $\pm$ 3.60	-2.43	0.015
	Urban	717	30.24 $\pm$ 3.33		
	All	1203			
HC (cm)	Rural	485	32.04 $\pm$ 3.82	-2.61	0.009
	Urban	715	32.60 $\pm$ 3.45		
	All	1203			

BW; Birth weight, BL; Birth Length, FL; Foot Length, FB; Foot Breadth, TC; Thigh Circumference, HL; Hand Length, HB; Hand Breadth, NAC; Neonatal Arm Circumference, CC; Chest Circumference, HC; Head Circumference, SD; Standard Deviation

Table 3: The effect of urbanisation on neonatal anthropometric parameters among the male neonates in Kano State

Sex	Variables	Domicile	N	Mean $\pm$ SD	t-value	P-value
Male	BW (g)	Rural	232	2972.41 $\pm$ 440.11	-5.71	1.85 $\times$ 10 ⁻⁸
		Urban	323	3203.41 $\pm$ 490.55		
	BL (cm)	Rural	232	50.10 $\pm$ 3.20	3.55	4.11 $\times$ 10 ⁻⁴
		Urban	323	49.07 $\pm$ 3.47		
	FL (cm)	Rural	232	8.31 $\pm$ 0.59	2.55	0.011
		Urban	323	8.17 $\pm$ 0.68		
	FB (cm)	Rural	232	3.15 $\pm$ 0.37	-1.98	0.048
		Urban	323	3.22 $\pm$ 0.40		
	TC (cm)	Rural	232	14.92 $\pm$ 2.09	0.45	0.656
		Urban	323	14.84 $\pm$ 1.99		
	HL (cm)	Rural	232	6.88 $\pm$ 0.44	1.81	0.071
		Urban	323	6.80 $\pm$ 0.55		
	HB (cm)	Rural	232	3.46 $\pm$ 0.31	0.97	0.333
		Urban	323	3.44 $\pm$ 0.31		
	NAC (cm)	Rural	232	11.11 $\pm$ 1.37	1.36	0.175
		Urban	323	10.95 $\pm$ 1.35		
	CC (cm)	Rural	232	29.73 $\pm$ 3.42	-1.37	0.172
		Urban	323	30.11 $\pm$ 3.12		
	HC (cm)	Rural	231	32.01 $\pm$ 3.66	-1.73	0.084
		Urban	321	32.52 $\pm$ 3.17		

BW; Birth weight, BL; Birth Length, FL; Foot Length, FB; Foot Breadth, TC; Thigh Circumference, HL; Hand Length, HB; Hand Breadth, NAC; Neonatal Arm Circumference, CC; Chest Circumference, HC; Head Circumference. SD; Standard Deviation

Table 4: The effect of urbanisation on neonatal anthropometric parameters among the female neonates in Kano State

Sex	Variables	Domicile	N	Mean $\pm$ SD	t-value	P-value
Female	BW (g)	Rural	254	2907.87 $\pm$ 418.66	-6.24	7.80×10^{-10}
		Urban	394	3151.02 $\pm$ 521.80		
	BL (cm)	Rural	254	48.92 $\pm$ 3.21	2.09	0.037
		Urban	394	48.38 $\pm$ 3.25		
	FL (cm)	Rural	254	8.12 $\pm$ 0.66	0.61	0.544
		Urban	394	8.09 $\pm$ 0.65		
	FB (cm)	Rural	254	3.08 $\pm$ 0.36	-3.15	0.002
		Urban	394	3.18 $\pm$ 0.42		
	TC (cm)	Rural	254	14.61 $\pm$ 2.16	-0.77	0.443
		Urban	394	14.74 $\pm$ 2.06		
	HL (cm)	Rural	254	6.76 $\pm$ 0.50	0.35	0.724
		Urban	394	6.75 $\pm$ 0.53		
	HB (cm)	Rural	254	3.39 $\pm$ 0.32	-1.80	0.072
		Urban	394	3.44 $\pm$ 0.36		
	NAC (cm)	Rural	254	11.01 $\pm$ 1.37	0.29	0.775
		Urban	394	10.98 $\pm$ 1.35		
	CC (cm)	Rural	254	29.77 $\pm$ 3.76	-2.00	0.046
		Urban	394	30.35 $\pm$ 3.50		
	HC (cm)	Rural	254	32.07 $\pm$ 3.97	-1.94	0.053
		Urban	394	32.66 $\pm$ 3.66		

BW; birth weight, BL; Birth Length, FL; Foot Length, FB; Foot Breadth, TC; Thigh Circumference, HL; Hand Length, HB; Hand Breadth, NAC; Neonatal Arm Circumference, CC; Chest Circumference, HC; Head Circumference. SD; Standard Deviation

Conclusion

It has been seen that in all the parameters considered, urban parameters were higher than those obtained among the rural populace. Therefore, in any strategic health planning, location (urban or rural) should be considered. This is in appreciation of the fact that location is a factor that can influence the outcome of the plan. Therefore, this implies that the urban/rural location of centres for data collection is a factor that influences the neonatal BW with the other neonatal and maternal anthropometric parameters.

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Association between Blood Pressure with Fingerprint and Palm Prints of Hausas of Kano and Zaria, Nigeria

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Abstract

Dermatoglyphics, the study of dermal ridges on finger balls, palms, and soles, with its qualitative and quantitative traits that are not adaptive and free from paratypic influences, provide a pertinent area of research to human population, geneticists interested in studying variation within and between populations. The aim of the study is to analyse fingerprints (pattern) and palm prints (palm creases) variables with blood pressure among Hausa of Kano and Zaria. A total of 600 subjects comprising of 300 participants from two original Hausa states (Kano and Zaria) with 1:1 male to female ratio participated. Ink method was used to capture fingerprint and palm print, and digital sphygmomanometer was used to measure blood pressure. Chi-square was used to determine the association between the variables. The result showed a significant association between the right thumbprint with diastolic blood pressures ($\chi^2 = 12.07$, $P < 0.05$) between the variable in Hausas of Kano state. However, no significant association was observed between left thumb-prints and systolic blood pressure in both states. A significant association ($\chi^2 = 11.90$, $P < 0.05$) was only observed among the Hausas of Kano state between left thumb-prints and diastolic blood pressure. In all the frequency of fingerprint patterns in both hands, left hand tends to be associated with diastolic blood pressure among Hausas of Kano state only. Comparison of association of thumbprint pattern and palmar creases type show no significant association between the two variables in both the Hausa state. Normal palm crease type happened to have high frequency of occurrence with respect to various fingerprint patterns. In conclusion, Hausas from Kano tend to show significant association with the variables than Hausas of Zaria despite the fact that they are of the same origin. This may be due to environmental factors.

Keywords: Blood pressure, Fingerprints, Palm prints, Hausas, Kano, Zaria

Introduction

Dermatoglyphics, the study of dermal ridges on finger balls, palms, and soles, with its qualitative and quantitative traits that are not adaptive and free from paratypic influences, provide a pertinent area of research to human population geneticists interested in studying variation within and between populations. [1,2] Studies shown that dermatoglyphics is used in establishing ethnic differences, genetic inheritance, and population studies respectively. [3,4,5]

Previous studies reported that hypertensive patients had a somewhat lower frequency of fingertip ulnar loops, higher frequency of whorls, higher ridge counts and higher mean atd angle. [6,7] Another study [8] demonstrated both fingertip whorls and narrow palmar angles are associated with high blood pressure in adult life. The incidence of double loop whorl pattern and arch are 4.57 % and 5.79 %

respectively in hypertensive and just 0.44 % and 1.33 % in normotensives. Though the differences of incidences of whorl as well as an ulnar loop between two groups are not so evident, but still statistically significant. [9] It was also observed that there was no association between the print pattern and hypertension in the males, while for the females there was significant association. This shows that the total fingerprint patterns for right and left hands for both sexes have similar patterns as above. For the combined group, there was significant association between the print pattern and hypertension and this is attributable to the very high association by the females. [10]

The Hausa ethnic group is one of Nigeria's largest ethnic groups, but data on their human biology is scarce. The present study intends to describe the

nature of digital dermatoglyphics pattern and its association with blood pressure across the two Hausa population as historically defined by the groups.

There is paucity of anthropological data on the ethnic groups in Nigeria especially the Hausa ethnic group. Thus, the objective of the study was to investigate association of fingerprint patterns with sex and blood pressure among the Hausa of Kano and Zaria, Nigeria.

Materials and Methods

Study Location and Population

The study was conducted in the two Hausa cities which comprises of Kano and Zaria, which is the north-west of Nigeria. Data for this study were collected from participants after informed consent was obtained from those who were willing to participate in the Survey, using a stamp pad, printer's duplicating ink, microscopic slide, digital sphygmomanometer, menthylated spirit, Cotton-wool and white plain sheets of paper. A total of six hundred (600) participants were selected using systematic random sampling method with a sex ratio of 1:1.

Fingerprint Collection

The participants were asked to wash and dry their hands and a traditional method of touching the ink pad with the tip of the finger and making an imprint on a white sheet of paper was used in collecting the fingerprint and the patterns were studied and recorded accordingly

Blood Pressure Measurement

For blood pressure measurement, a digital sphygmomanometer was used. The subjects were asked to sit down, calmed and relaxed on a chair. The cuff was wrapped well on the left arm which lies close to the participant's heart then the start

button was pressed, and the readings were taken thrice, and the average of the closest two readings was taken. The blood pressure categorization was according to WHO classification. ^[11] For systolic blood pressure; optimal < 120, normal 120-139 and hypertensive 140-180 and for diastolic blood pressure; optimal < 80, normal 80-89 and hypertensive 90-110.

Statistical Analyses

Data were reported using descriptive statistics of frequency/ percentages. A Chi-square test was used to test for association between qualitative variables. $p < 0.05$ was deemed statistically significant and SPSS version 22 (IBM, Corp., New York) was used for the data analysis.

Result

In Tables 1 and 2 the frequency distribution of the fingerprints pattern on the 10 digits of both males and females Hausas of Kano showed a high-frequency occurrence of loops followed by whorls, then arches recorded the least. A high occurrence of loops pattern was observed on the little finger of the right (110 % and 111 % in male and female respectively) and left (122 % and 111 % in male and female respectively) hands. Statistically significant association ($\chi^2 = 8.26$, $p = 0.016$) was observed only on the left third digit among Hausa populations of Kano state. Frequency distribution of the finger pattern on the 10 digits of both males and females Hausas of Zaria showed similar trend of predominance in pattern of fingerprints. However, a statistically significant association ($\chi^2 = 7.32$, $p = 0.021$) was noticed on the left second digit (Tables 3 and 4). It was also noticed that Hausas from Kano tend to have a higher frequency occurrence of loops pattern in all digits of both hands in males and females than the Hausas of Zaria. In both sexes, loops exhibited high frequency of occurrence while arches were the least for both states.

Table 1: Frequency distribution of fingerprint pattern on the right five digits of Hausa of Kano (n = 150 in both males and females)

Digits	Sex	Loops (%)	Whorls (%)	Arches (%)	χ^2	P
Thumb(1D)	Male	57(64.0)	80(71.0)	13(15.0)	4.35	0.114
	Female	71(64.0)	62(71.0)	17(15.0)		
Index(2D)	Male	84(85.5)	50(47.0)	16(17.5)	4.35	0.112
	Female	87(85.5)	44(47.0)	19(17.5)		
Middle(3D)	Male	104(106.5)	30(27.5)	16(16.0)	0.572	0.751
	Female	109(106.5)	25(27.5)	16(16.0)		
Ring(4D)	Male	91(89.0)	48(47.5)	11(13.5)	1.026	0.599
	Female	87(89.0)	47(47.5)	16(13.5)		
Little(5D)	Male	110(110.5)	34(31.5)	6(8.0)	1.401	0.496
	Female	111(110.5)	29(31.5)	10(8.0)		

Table 2: Frequency distribution of fingerprint pattern on the left five digits of Hausa of Kano (n = 150 in both males and females)

Digits	Sex	Loops	Whorls	Arches	χ^2	P
Thumb(1D)	Male	70(66.0)	68(68.0)	12(16.0)	2.485	0.289
	Female	62(66.0)	68(68.0)	20(16.0)		
Index(2D)	Male	84(79.5)	46(46.5)	20(24.0)	1.854	0.396
	Female	75(79.5)	47(46.5)	28(24.0)		
Middle(3D)	Male	105(99.5)	33(30.0)	12(20.5)	8.257	0.016
	Female	94(99.5)	27(30.0)	29(20.5)		
Ring(4D)	Male	98(96.0)	42(39.0)	10(15.0)	3.878	0.144
	Female	94(96.0)	36(39.0)	20(15.0)		
Little(5D)	Male	122(116.5)	22(23.5)	6(10.0)	3.911	0.142
	Female	111(116.5)	25(23.3)	14(10.0)		

Table 3: Frequency distribution of fingerprint pattern on the right five digits of Hausa of Zaria (n = 150 in both males and females)

Digits	Sex	Loops	Whorls	Arches	χ^2	P
Thumb(1D)	Male	55(57.7)	84(80.3)	11(12.0)	0.781	0.677
	Female	60(57.3)	76(79.7)	13(12.0)		
Index(2D)	Male	67(64.2)	65(66.2)	18(19.6)	0.539	0.764
	Female	61(63.8)	67(65.8)	21(19.4)		
Middle(3D)	Male	94(91.8)	38(40.6)	18(17.6)	0.470	0.790
	Female	89(91.2)	43(40.4)	17(17.4)		
Ring(4D)	Male	77(74.7)	69(67.7)	4(7.5)	3.498	0.174
	Female	72(74.3)	66(67.3)	11(7.5)		
Little(5D)	Male	94(93.8)	50(48.7)	6(7.5)	0.695	0.707
	Female	93(93.2)	47(48.3)	9(7.5)		

Table 4: Frequency distribution of fingerprint pattern on the left five digits of Hausa of Zaria (n = 150 in both males and females)

Digits	Sex	Loops	Whorls	Arches	χ^2	P
Thumb(1D)	Male	67(63.7)	69(72.2)	14(14.0)	0.623	0.729
	Female	60(63.3)	75(71.8)	14(14.0)		
Index(2D)	Male	78(67.2)	48(59.2)	24(23.6)	7.732	0.021
	Female	56(66.8)	70(58.8)	23(23.4)		
Middle(3D)	Male	91(88.3)	40(43.6)	19(18.1)	0.876	0.645
	Female	85(87.7)	47(43.4)	17(17.9)		
Ring(4D)	Male	82(83.8)	56(55.7)	12(10.5)	0.488	0.783
	Female	85(83.2)	55(55.3)	9(10.5)		
Little(5D)	Male	106(103.8)	36(39.1)	8(7.0)	0.865	0.649
	Female	101(103.2)	42(38.9)	6(7.0)		

Figure 1 compared the right thumbprint with systolic blood pressure which showed no association ($p > 0.05$) in both Hausa cities. Whereas in Figure 2 which compared right thumbprint with diastolic blood pressure showed a significant association ($\chi^2 = 12.07$, $P < 0.05$) between the variable in Hausas of Kano state in Figure 3 no significant association was observed in both cities between the left thumb-prints and systolic blood pressure. A significant association ($\chi^2 = 11.90$, $P < 0.05$) was observed among the Hausas of Kano state between left thumb-prints and

diastolic blood pressure (Figure 4). In all, the frequency of fingerprint patterns in both right and left hand tends to be associated with diastolic blood pressure among Hausas of Kano state only. From Figures 5, 6, 7 and 8 comparison of association of thumbprint pattern and palmar creases type showed no significant association between the two variables in both the Hausa state. Normal palm crease type happened to have high frequency of occurrence with respect to various fingerprint patterns.

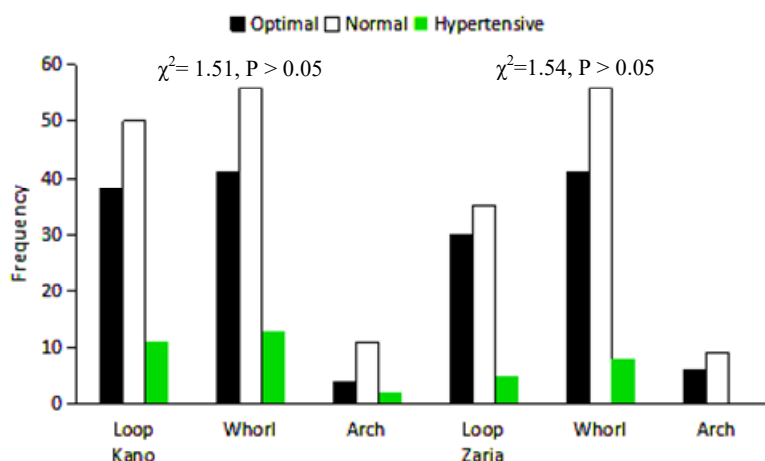


Fig. 1: Association of right thumb-prints and systolic blood pressure between Hausas of Kano and Zaria (optimal < 120, normal 120 - 139 and hypertensive 140 - 180).

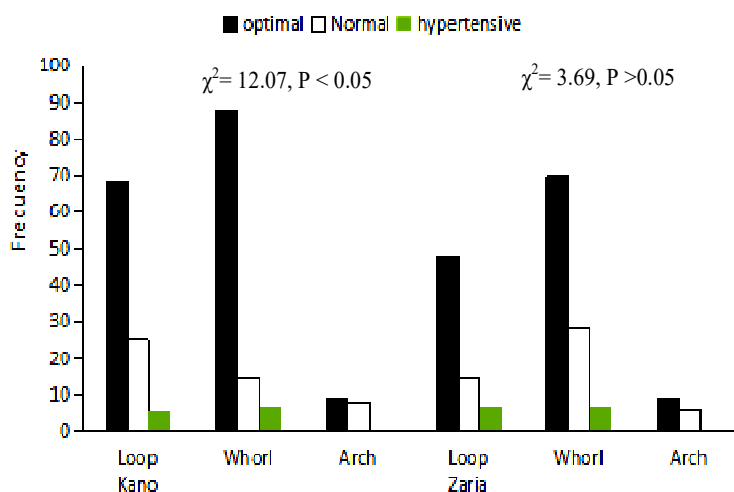


Fig. 2: Association of right thumb-prints and diastolic blood pressure between Hausas of Kano and Zaria (optimal < 80, normal 80-89 and hypertensive 90 - 110).

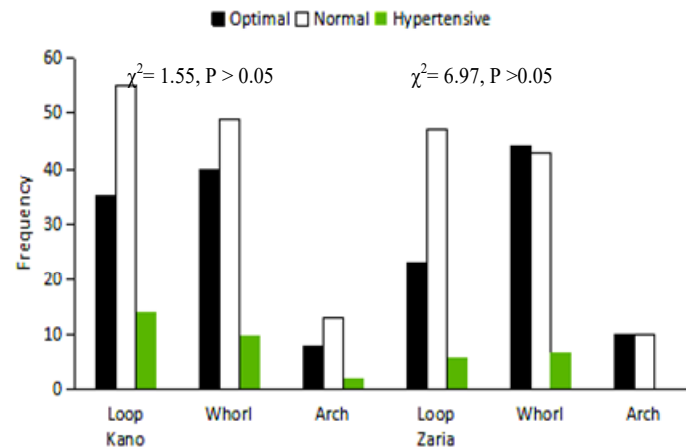


Fig. 3: Association of left thumb-prints and systolic blood pressure between Hausas of Kano and Zaria (optimal < 120, normal 120 - 139 and hypertensive 140 - 180).

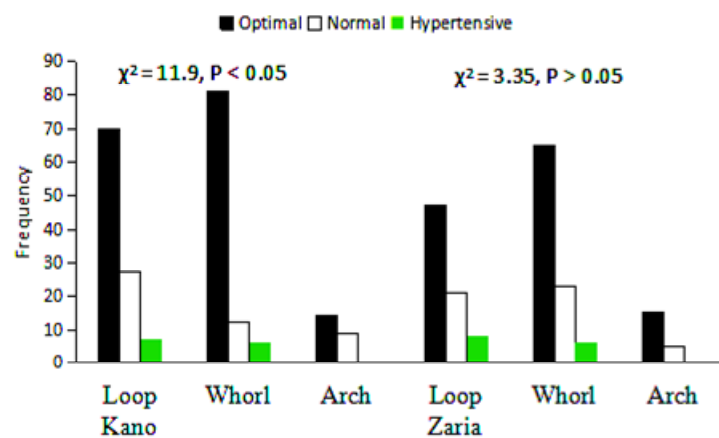


Fig. 4: Association of left thumb-prints and diastolic blood pressure between Hausas of Kano and Zaria (optimal < 80, normal 80 - 89 and hypertensive 90 - 110).

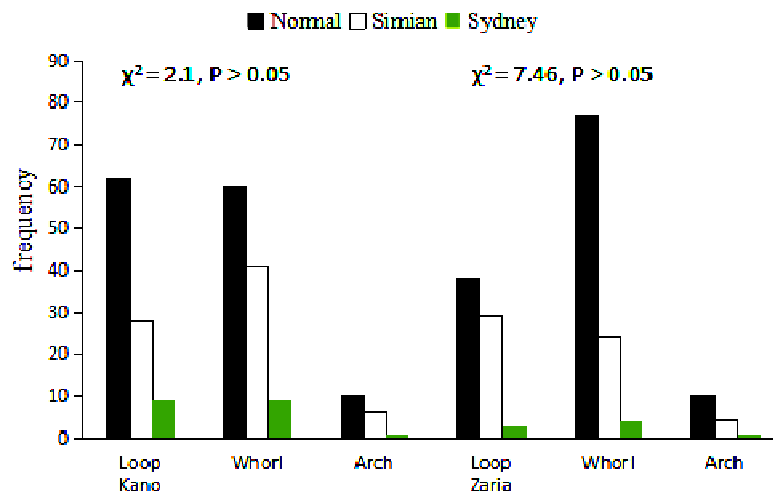


Fig. 5: Association of right thumb-prints and right palm crease between Hausas of Kano and Zaria

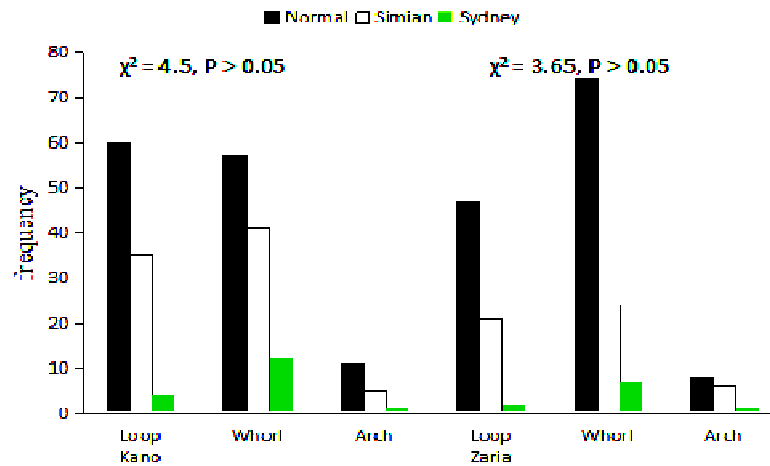


Fig. 6: Association of right thumb-prints and left palm crease between Hausas of Kano and Zaria

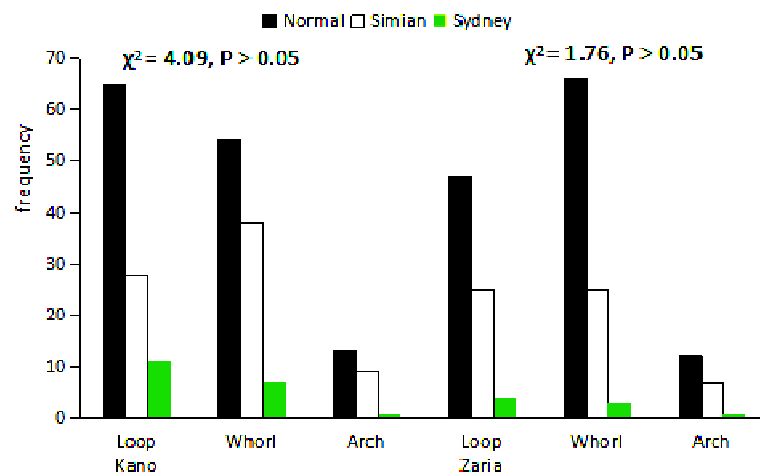


Fig. 7: Association of left thumb-prints and right palm crease between Hausas of Kano and Zaria

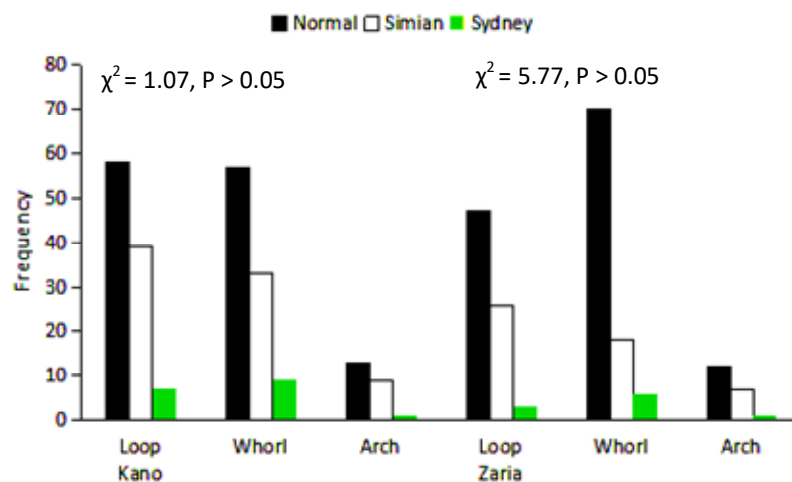


Fig. 8: Association of left thumb-prints and left palm crease between Hausas of Kano and Zaria

Discussion

The present study presented the frequency distribution of the pattern of fingerprints or Hausas of Kano and Zaria cities in which loops showed the highest occurrence followed by whorls, then arches. This follows universal pattern of Henry system of classification, in which there are three basic fingerprint patterns: Loop, Whorl and Arch, constituting 60-65%, 30-35% and 5% of all fingerprints respectively. [12] Although, in the previous studies of fingerprint pattern distribution of Hausa ethnic group no attempt to differentiate the ethnic groups based on the original Hausa cities but still the universal pattern of loop exhibiting the highest frequency and arches the lowest was noticed. [5] This indicated that the universality of the pattern is constant throughout the population of the world, Hausa inclusive.

With regards to sexual dimorphism in the fingerprints patterns, it was observed that statistically, a significant association existed between the sexes only on the left third digit in Hausa of Kano and the left second digit for Hausas in Zaria. The findings agreed with the previous findings. [5] This is representing typical pattern of fingerprints pattern of the Hausa in general. However, disturbance in the pattern of the frequency was observed after grouping the Hausa based on the two different Hausa states. This may be considered as one of the justifications for the need of considering Hausa based on the original Hausa states in case of anthropometric measurements that is sensitive to ethnicity.

Another digital dermatoglyphics study of two ethnic groups in Nigeria, the Yoruba and the Igbo stated that only the Igbo showed a significant gender difference in the rather unusual direction with the male having fewer whorls and more arches than the females. [13] Analysis of the fingerprints of the Tibetans showed that whorls were more abundant than loops in males. However, females exhibited a much frequency of whorl and a higher frequency of loop. [14] This may be additional indices that can be used to provide distinction between sex and different population across the world.

Several studies have provided evidence that dermatoglyphic traits are associated with hypertension. Previous studies reported that

hypertensive patients had a somewhat lower frequency of fingertip ulnar loops, higher frequency of whorls. [6, 7] In the present study comparison of right thumbprint with systolic blood pressure showed no association in both Hausa population of Kano state and Zaria city whereas for the right thumbprint and diastolic blood pressure the fingerprint pattern in both right and left hand tend to be associated with diastolic blood pressure among Hausas of Kano state only. This may explain the role of environmental factors and lifestyle in the association. Despite the two populations are of the same ethnic group but of course exposed to same environmental condition. The lifestyle also differed between the populations. It can, therefore, be suggested that the existence of association between fingerprints pattern and blood pressure is multi-factorial in nature.

This result is similar to those done on other populations. For example, Pursnani *et al.* [8] demonstrated that both fingertip whorls are associated with high blood pressure in adult life. The incidence of the double loop, whorl pattern and arch are higher in hypertensive compared to normotensives. Though the differences of incidences of whorl as well as ulnar loop between two groups are not so evident, but still statistically significant. [9] A report indicating a strong correlation between a rise in whorl frequency with a rise in blood pressure. [15]

Moreover, gender-wise, analyses observed that there was no association between the fingerprint pattern and hypertension in the males, while for the females there was significant association. It was reported that the total fingerprint patterns for right and left hands for both sexes show similar patterns as above. For the combined group, there was significant association between the print pattern and hypertension which could be attributable to the very high association by the females. [10] Similarly, in our study, Hausa state-wise comparison between fingerprint pattern and blood pressure indicated that the significant association was observed only in Hausas of Kano state. This may provide further insight in segregation of the two Hausa ethnic groups somewhere along genetic lineage. It was suggested that since other variables used to associate blood pressure were either unique or not found

across several studies, it seems prudent at this time to afford them less weight with respect to determining whether or not certain dermatoglyphic features can be associated with hypertension.^[16] The finding from this study added the few data on the human biology of the Hausa ethnic groups. It also contributes to providing the overall picture of the biological traits of the Nigerian population.

In conclusion, the finding of the present study presents that the frequency distribution of the pattern of fingerprints of Hausas of Kano and Zaria state. The significant sexual dimorphism in the frequency of fingerprint pattern was observed on the left digits of the two Hausa population. Fingerprint pattern in both right and left hand tends to be associated with diastolic blood pressure among Hausas of Kano state only

Conflict of interest:

None declared by authors.

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Study of the Effects of Methanolic Extract of *Momordica charantia* on Histo-Morphology and Enzyme Concentration of the Liver of Adult Albino Rats

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Abstract

This study was aimed at determining the effects of the extract of *Momordica charantia* on the histo-morphology and enzyme concentration of the liver of albino rats. A total of 20 adult male albino rats of mean weight of 270 ± 30 gram was used. The animals were grouped into four groups, A, B, C and D which received distilled water, 125 mg/kg, 250 mg/kg and 500 mg/kg body weight respectively. LD₅₀ of *Momordica charantia* was determined to be 1855 mg. The administration period was six weeks; weights of the rats were recorded weekly. Histological slides of liver tissue were determined using standard procedures. The histology of the liver of rats treated with 125 mg/kg body weight of methanolic extract *Momordica charantia* showed some vacuolation of hepatocytes, necrosis at some areas and dilated hepatic cords. This study revealed that there were more lesions in the liver of rats which were administered with 250 mg/kg and 500mg/kg body of the methanolic extract of *Momordica charantia*. Where there were more focal areas of necrosis and more vacuolated hepatocytes and dilated hepatic cords in dose-dependant manner. There was increase in concentration of liver enzyme across the groups treated with extract of *Momordica charantia* when compared to the control group. In conclusion, hepatic vacuolation and necrosis were observed in the treated groups as opposed to normal architecture of liver cells in the control group. The effects of the extract were observed to be dose dependent.

Keywords: Liver, Histomorphology, Enzyme, *Momordica charantia*, Albino rats

Introduction

Momordica charantia is widely grown in tropical areas such as the Amazon, East Africa, Asia, India, West Africa, and Caribbean. It is used traditionally as both food and medicine.¹ The plant is a climbing perennial with elongated fruit (the cultivated type) and or small rounded fruits (the wild type). The elongated fruit resembles a warty gourd or cucumber. The unripe fruit is white or green in colour and has a bitter taste that becomes more pronounced as the fruit ripens. In the northern part of Nigeria, flowering occurs during the months of June and July, while fruiting occurs during the months of September to November.² The fruits have a distinct watery exterior and an oblong shape at both ends. It is hollow in cross-section with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds.

The clinical conditions for which *Momordica charantia* extracts (primarily from the fruits) are currently being used include: diabetes mellitus, dyslipidemia, microbial infection and potentially as a cytotoxic agent for certain types of cancer.³ The plant has numerous other constituents such as proteins glycosides, saponins and minerals like vitamins A and C and beta-carotene, as well as the minerals iron, phosphorus and potassium.⁴ Several animal studies using a rodent model of diabetics have examined the effect of bitter melon extracts on abnormal lipid parameters. Significant decreases in triglycerides and low diet lipids (LDL) cholesterol and increases in High Diet Lipid (HDL) cholesterol were noted.^{5, 6, 7}

Traditional medicine in Nigeria and other developing countries where conventional medical

facilities cannot satisfy their national demands plays an important role in combating both human and animal diseases. It was estimated that about 80% of the people who live in rural areas, rely on the traditional medicine healers for the treatments of their health problems, using medicinal herbs.⁸ However, reports on the effects of *Momordica charantia* on the histo-morphology of liver are scanty or received less attention. Most of the information is still in the hands of the traditional healers, therefore this study becomes necessary. This study was aimed at determining the effects of the extract of *Momordica charantia* on the histo-morphology and enzyme concentration of the liver of albino rats after administration of methanolic extract of *Momordica charantia*.

Materials and Methods

Experimental Animals

Twenty male adult albino rats with a mean body weight of 270 ± 30 g were purchased from Animal House of Human Anatomy Department, Ahmadu Bello University, Zaria. The rats were randomly selected and used for this study. They were grouped into a control group (group **A**) and treatment groups (groups **B**, **C**, and **D**), according to their body weights (Table 1). They were kept in the Animal House of Anatomy Department, Ahmadu Bello University, Zaria for two weeks to acclimatized with the environment according to Grover *et al.*⁹ During the acclimatization period, the animals were closely monitored and standard environmental conditions $\pm 25^\circ\text{C}$ was maintained, relative humidity of 60% and 12 hours light and dark cycle were maintained. The animals were properly fed with growers feed on vital feed company. The animals were housed in plastic cages with stainless steel mesh covers, the floors were covered with sawdust which soaked away the urine of the rats. This was changed on daily basis.

The plant

The plant was obtained from some gardeners around Shika Dam, Zaria Local Government Area in Kaduna State, Nigeria in the month of September. The plant was taken to the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, for identification and authentication. The plant was identified as follows: Family name: Cucurbitaceae, *Momordica charantia*, local name (Hausa): Garahuni, Common name, Balsam pear, Voucher number: 1139.

The collection of the plant took about two (2) weeks and was taken in the early morning and after sunset as was suggested by Sofowora.¹⁰ The leaves and stem of the plant were carefully packed into a well-ventilated room and spread sparsely for shade drying. Every two (2) days the spreading plant was checked and overturned. This process was run for two (2) weeks for the plant to dry. The leaves and stem of the plant were packed and crushed into powder by means of wooden mortar and pestle. The powdered form was weighed to be 2.6 kg which was assumed to be enough for the extraction procedures.

Extraction of *Momordica charantia*

The maceration method was used for the extraction; 500 g of the powder of the plant was taken and placed into a separating funnel with clean cotton wool placed at the narrowed part of the funnel to block the passage of the plant into the collecting beaker. At first, 2 litres of cool methanol was poured onto the 500g in the funnel and allowed to dry for 12 hours. The powder was observed to be greenish which suggested that there were more of plants' constituents remaining, hence the need to put more methanol. The extraction continued for another 12 hours until the powder became pale yellowish. A total of 5.5 litres of methanol was used for the methanolic extraction. The filtrate was collected in a collecting beaker and poured into evaporating dishes which were on water bath machines at 45°C . The filtrate was evaporated and dried for 8 hours. The filtrate was then collected in a glass bottle and weighed.

Phytochemical Screening of *Momordica charantia*

Basic phytochemical screening was carried out to detect the presence of alkaloids, tannins, saponins, anthraquinones, cardenolides in the plant's extract. Simple, standard chemical tests have been devised for this study as laid down by Evans¹¹ and adopted by Abdullahi *et al.*¹². A small portion of the extract about 0.5g was taken and dissolved in a test tube containing distilled water. A drop of 0.5 ml was taken for each of the tests tubes.

Acute Toxicity Study

The limit test of the extract of *Momordica charantia* was conducted at an as high dose as 1000 mg/kg body weight and was relatively found to be safe within 48 hours observation period following oral administration of a single dose to the adult albino rats. LD₅₀ was determined to be 1855 mg.^{11, 12} The

choice of the dosages was based on the fact that at high dose of 1855mg of the methanolic extract of *Momordica charantia*, there was no toxicity was observed in treated rats for pilot study. Therefore, the animals were exposed to the selected dosages for a period of six (6) weeks to induce chronic toxicity as was suggested by Grover and Yadav.¹³

Administration of the Extract to the Animals

The animals were weighed just before the commencement of the extract administration. The administration of the extract was done for a period of six (6) weeks. The extract of *Momordica charantia* was measured by means of electronic measuring machine which measures in milligram

(mg). The measured extracts were then transferred to well labelled small size plastic bottles. The bottles were labelled 125 mg, 250 mg and 500 mg of *Momordica charantia*. The corresponding weights were placed in each bottle with the same label.

The extract of *Momordica charantia* is water-soluble and was administered by oral route. Robber-capped needles were used to administer the extracts. The extracts were dissolved in warm distilled water by vigorous shaking, to facilitate dissolution of the extract. The content was immediately taken and administered to the rats. The period of extract administration took six (6) weeks (Table 1) and was done at a fixed time daily 9:00 am as was done by Atama and Idu.¹⁴

Table 1: Animal grouping, average weight, and extract administration scheme

Groups	N	Average weight	Administration		
			Dose	Route	Duration
A	5	-	Control	Oral	Six weeks
B	5	260±20 g	125 mg	Oral	Six weeks
C	5	258±20 g	250 mg	Oral	Six weeks
D	5	264±30 g	500 mg	Oral	Six weeks

Animal Sacrifice

The rats were humanely sacrificed and their liver was harvested and fixed in neutral buffered formalin solution for histo-morphological study.

Liver Enzyme Assay

The blood was carefully taken by cardiac puncture through the anterior surface of the heart, using sterilised syringes. About 5ml to 7ml was immediately transferred to the collecting bottles. The blood was centrifuged using centrifuging machine at 1000rpm and the serum obtained was used for biochemical analysis in Ahmadu Bello University, Teaching Hospital, Zaria, for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) as proposed by Sujathan *et al.*¹⁵

Statistical Analyses

The data obtained were analysed using SPSS software version 20. One-way analysis of variance (ANOVA) was used to compare data between the groups and Holm Sidak post hoc test was used for multiple comparisons. This test best compares the level of significance between the control and the

treated groups. All data were expressed as Mean±SD and P<0.05 were considered to be significant.

Results

The histology of the liver of the control group treated with distilled water showed normal hepatic architecture and no lesion was observed (Plates I and II). The histology of the liver of rats treated with 125 mg/kg body weight of methanolic extract *Momordica charantia* showed some vacuolation of hepatocytes, necrosis in some areas and dilated hepatic cords (Plate III and IV). This study revealed that there were more lesions in the liver of rats which were administered with 250 mg/kg body of the methanolic extract of *Momordica charantia*. There were more focal areas of necrosis and more vacuolated hepatocytes and dilated hepatic cords (Plates V and VI). It was also observed that there were more lesions in the liver of rats which were administered with 500 mg/kg body weight than the other groups administered with the lower doses. It showed more focal area of necrosis, more vacuolation of hepatocytes and more dilated hepatic cords (Plates VII and VIII)

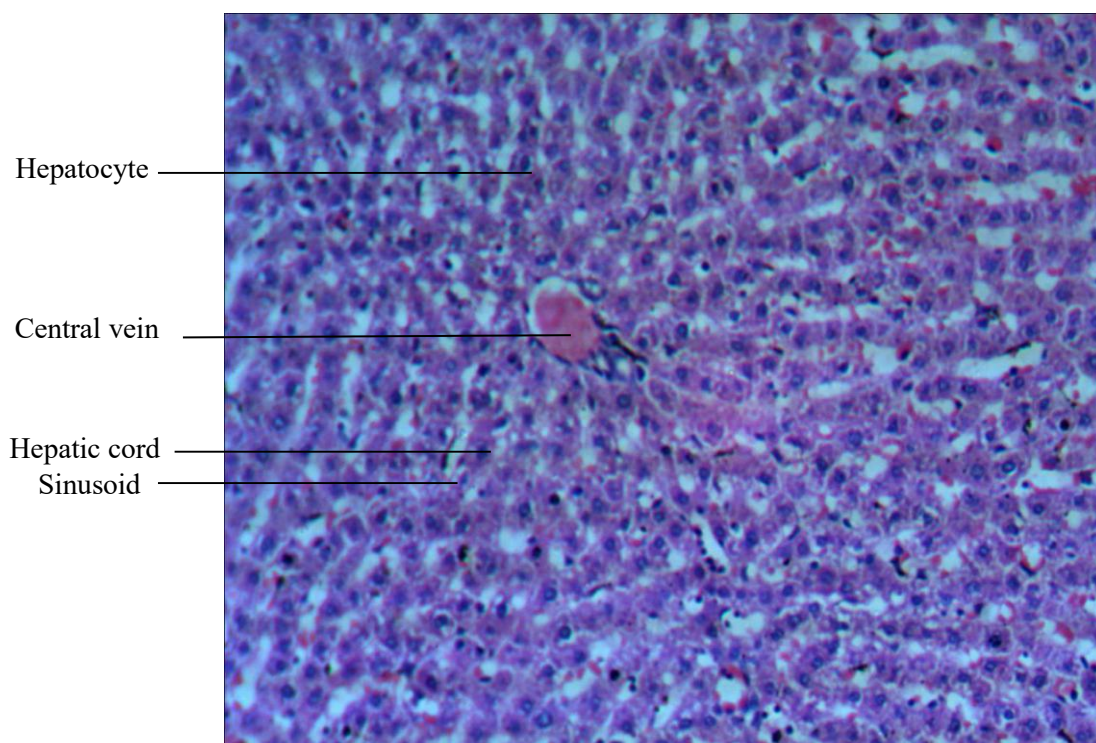


Plate I: A cross-section of the liver administered with distilled water (control group) showing normal histology (Hematoxyline and Eosin) $\times 250$

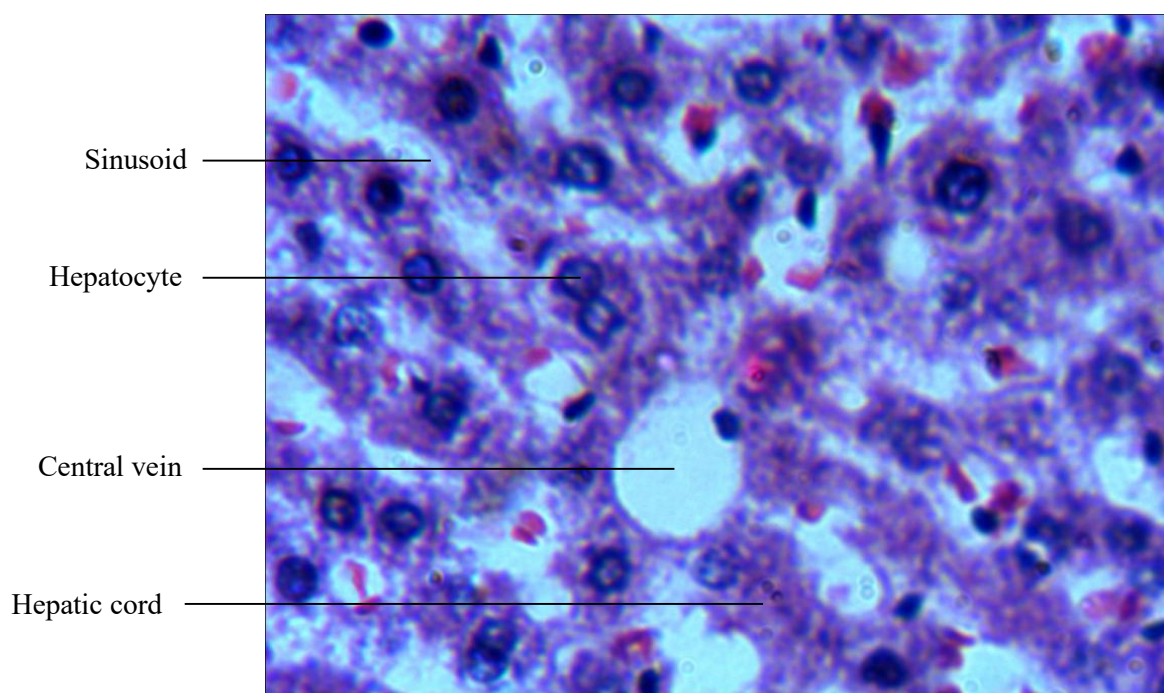


Plate II: A cross-section of the liver administered with distilled water (control group) showing normal histology (Hematoxyline and Eosin stain) $\times 400$

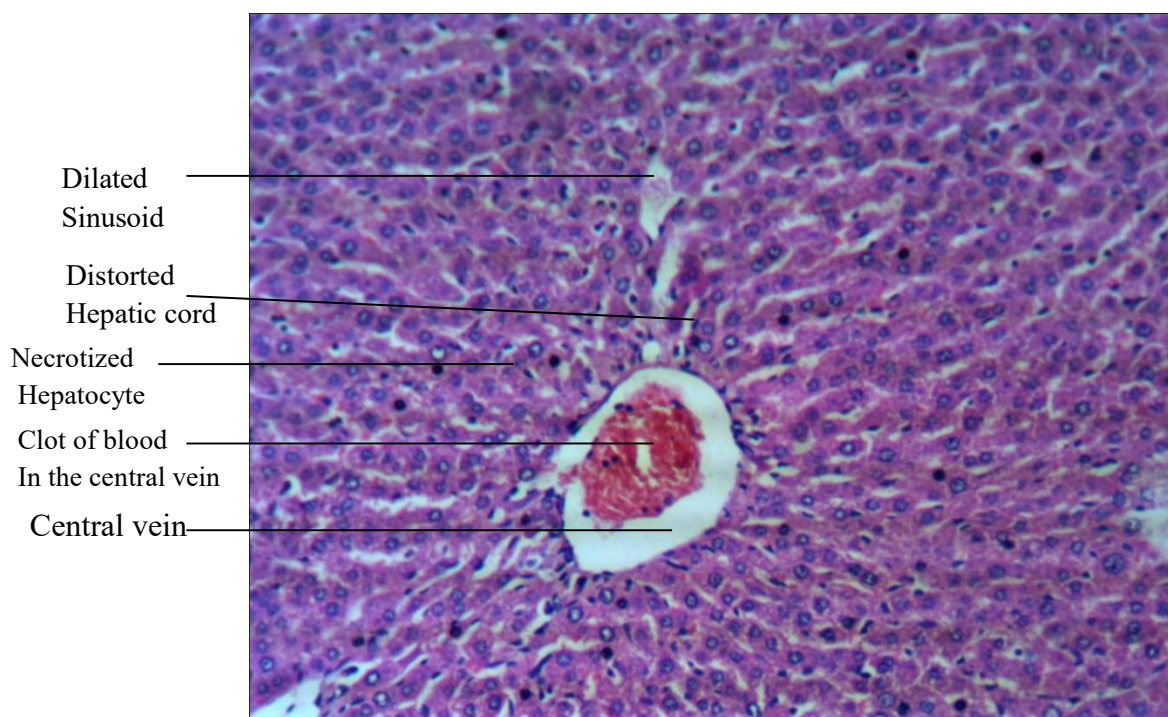


Plate III: A cross-section of the liver treated with 125mg/kg bodyweight of *Momordica charantia* showing dilated central vein, dilated sinusoid and distorted hepatic cords (Hematoxyline and Eosin) $\times 250$

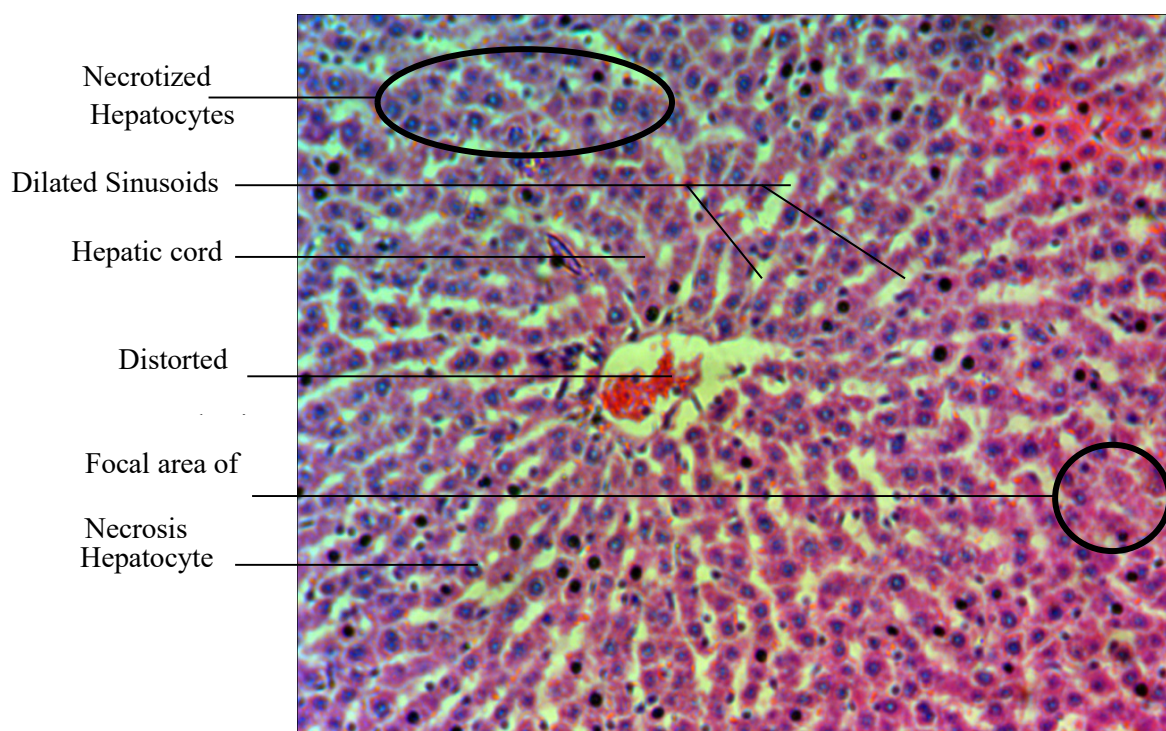


Plate IV: A cross-section of the liver treated with 125mg/kg bodyweight of *Momordica charantia* (methanolic extract) showing focal area of necrosis, dilated sinusoids and distorted central vein (Hematoxyline and Eosin) $\times 250$

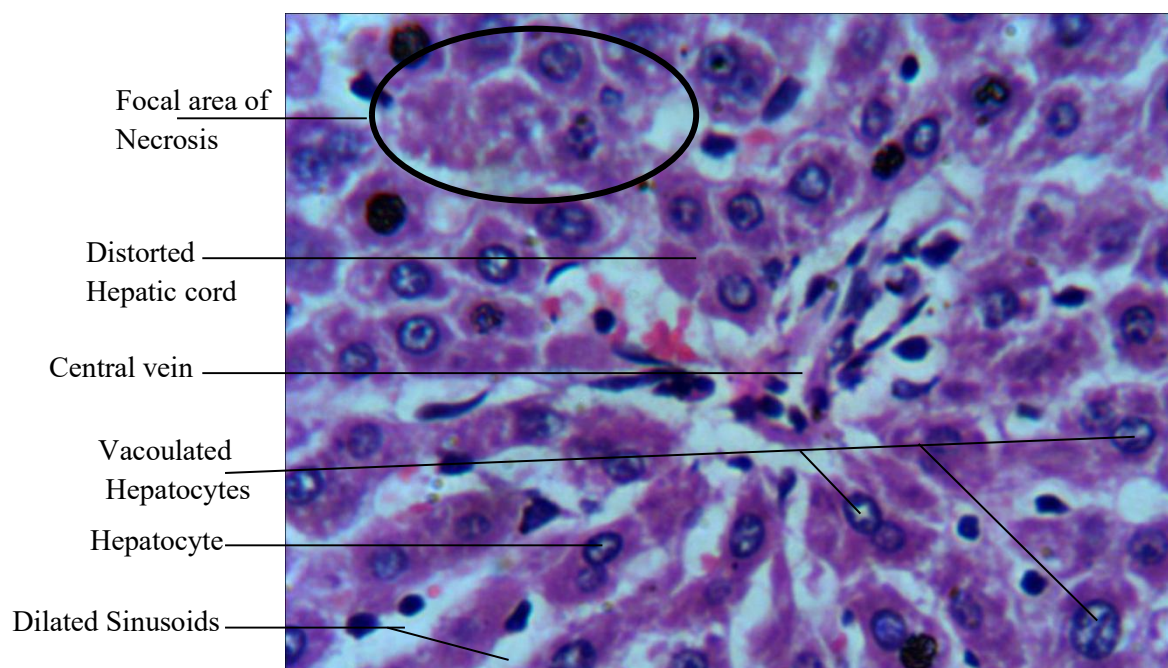


Plate V: A cross-section of liver treated with 125mg/kg bodyweight of *Momordica charantia* showing congested vacoulation of hepatocyte, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) $\times$ 400

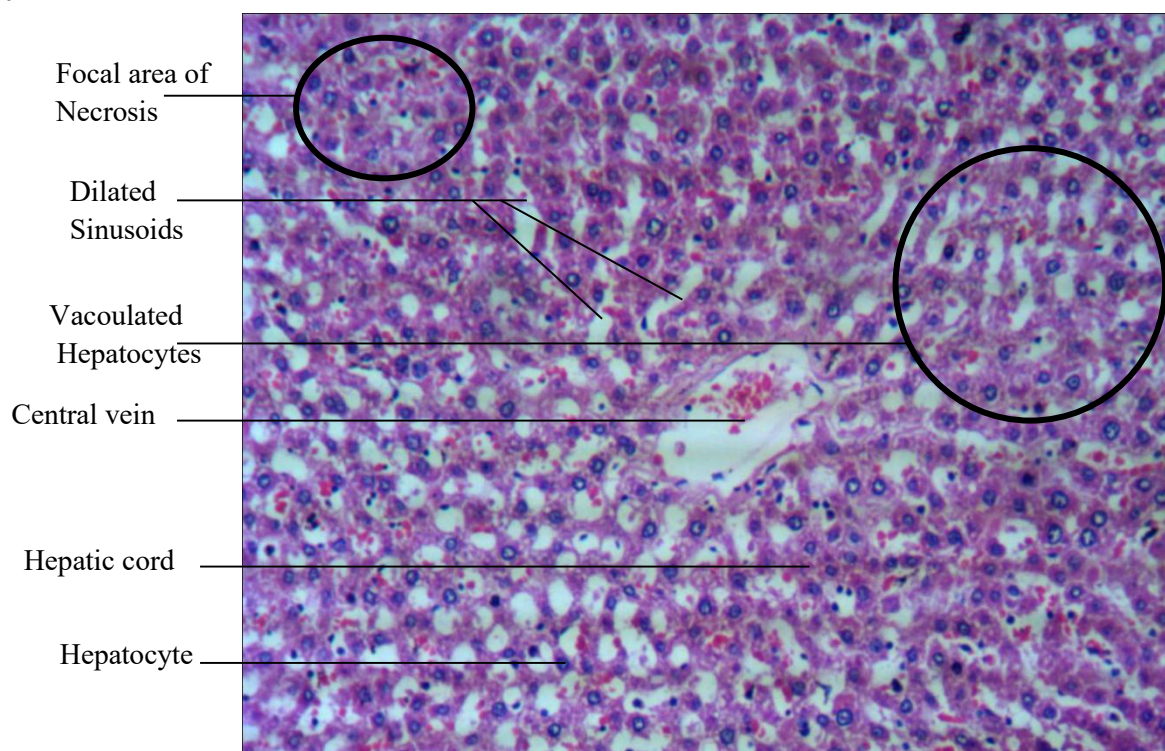


Plate VI: A cross-section of liver treated with 250mg/kg bodyweight of *Momordica charantia* showing area of vacoulation of hepatocytes, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) $\times$ 250

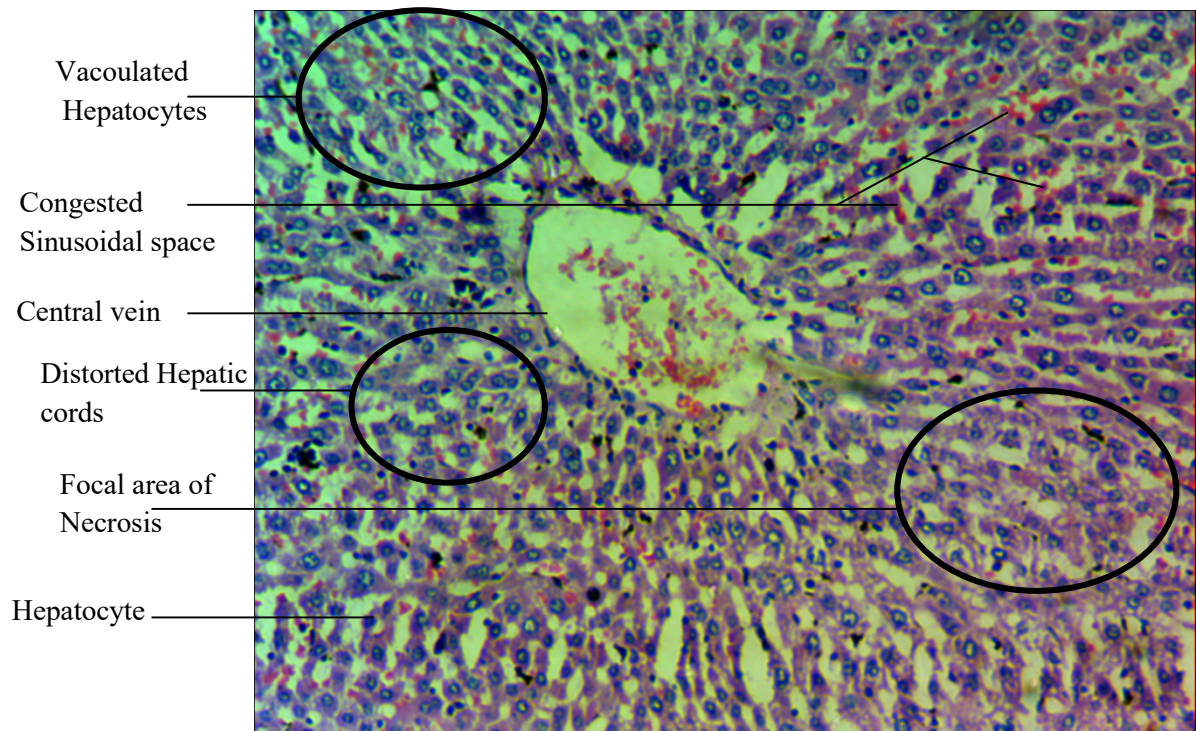


Plate VII: A cross-section of the liver treated with 500mg/kg bodyweight of *Momordica charantia* showing area of vacuolation of hepatocytes, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) $\times$ 250

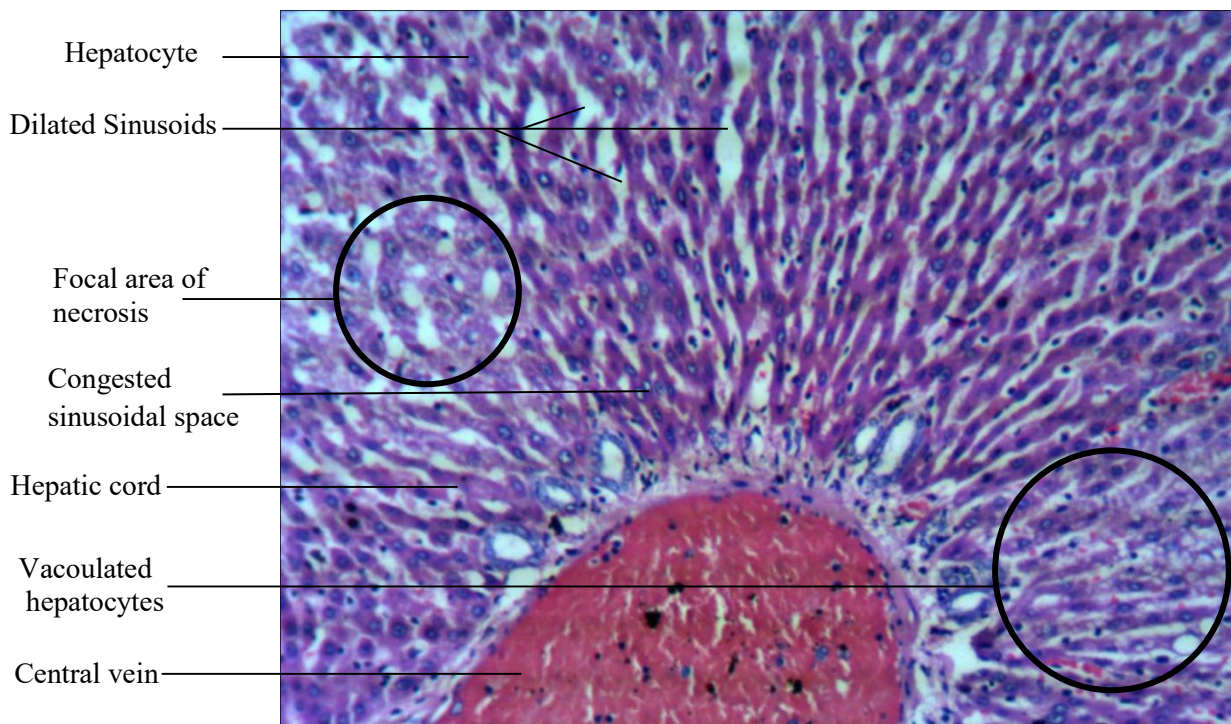


Plate VIII: A cross-section of liver treated with 500mg/kg bodyweight of *Momordica charantia* showing area of vacuolation of hepatocytes, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) $\times$ 400

The biochemical analysis of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in this study showed an increase in

concentration of these enzymes across the groups treated with extract of *Momordica charantia* when compared to the control group (Figure 1).

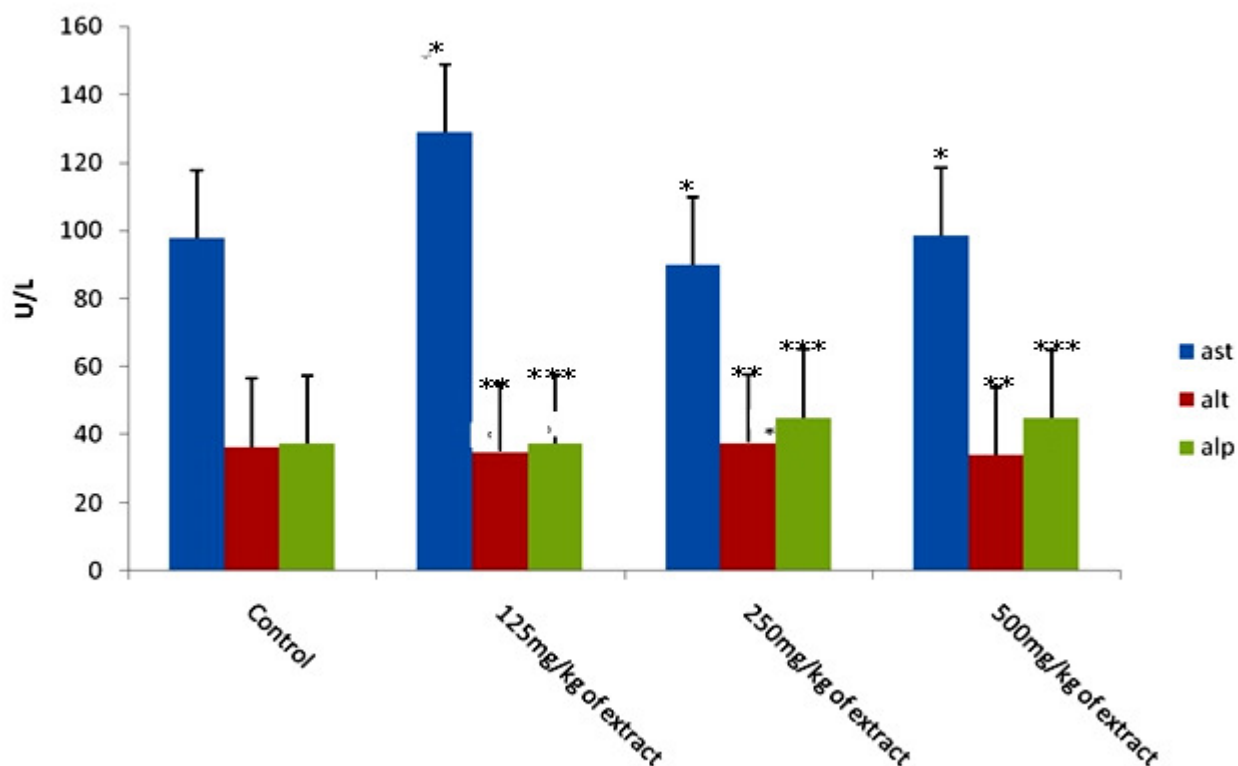


Fig. 1: Effect of methanolic extract of *Momordica charantia* on liver enzymes (AST, ALT and ALP) levels in albino rats (**P<0.008, ** P<0.036, *P<0.022).

Discussion

Plants have always been a very useful source of remedy to several ailments owing to its easy availability and affordability in traditional settings worldwide. Some of these plants that exhibit medicinal properties have been known to help in stabilizing different internal organs in humans and animals, while others have side effects on the organs probably due to the varying amounts or quantity of toxic matter present in such plants.¹⁶ The present study investigated the possible effects of the extract of *Momordica charantia* on the histo-morphology and enzyme concentration of the liver of albino rats after administration of methanolic extract of *Momordica charantia*.

It was observed that the methanolic extract of *Momordica charantia* administered to the rats in all the treatment groups has caused some mild to severe damage, for example, the group of rats which were administered with 125 mg/kg body weight had vacuolation of hepatocytes. The vacuolation of hepatocytes (also called ballooning degeneration) is hepatic damage caused by ingested toxic or immunologic insult to the liver. The hepatocytes become swollen, edematous in appearance with irregularly clumped cytoplasm and clear spaces like vacuoles within hepatocytes. This type of condition could be caused by toxic chemicals such as iron, copper, accumulation of fat droplets (known as

steatosis). Similar lesions were observed by Jungermann and Kietzmann.¹⁷

The focal areas of necrosis were also observed with dilated sinusoids, distorted hepatic architecture, and congested liver. In liver pathology, any significant insult to the liver may cause hepatocyte necrosis. It can be characterized by poorly stained mummified hepatocytes with sometimes lysed nuclei at some parts.¹⁸

In this study, it was found that the levels of enzymes such as AST, ALT, and ALP of the treated groups had increased levels when compared to the control group. The statistical analysis of their values showed that there was marked elevation of AST among the groups which were treated with the extract of *Momordica charantia*. The effects of the extract are dependent. The group that received the highest dosage of 500mg/kg body weight had higher levels of AST, ALT, and ALP. This suggests that there were more assaults on the liver, as these enzymes are biomarkers of liver disease. This conforms to the histological changes observed in this study. Similar findings were reported in the previous study.¹⁹

AST and ALT are the liver enzymes, which are biomarkers of liver health. When these two enzymes are found to be elevated instead of ALP, it is an indication of liver cell necrosis. When ALP is elevated, a problem with bile flow is most likely to occur. This problem leads to the elevation of bilirubin. This condition is associated with severe liver disease or gall bladder disease. Therefore, once there is an elevation of ALP, bilirubin test can be useful in determining liver function.

In conclusion, hepatic vacuolation and necrosis were observed in the treated groups as opposed to the normal architecture of liver cells in the control group. The extract also caused increased level of liver enzyme. Hence, the oral administration of extract of *Momordica charantia* has produced some negative effects on histomorphology and enzyme concentration of the liver of adult albino rats.

Conflict of Interest: None

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Effect of Administration of Aqueous Extract of *Moringa Oleifera* Lam Leaves on the Histo-Architecture of Testes of Male Adult Wistar Rats

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Abstract

Moringa oleifera has various nutritional benefits and uses in many fields including industries. However, it is important to note that most investigations on this plant are basic and the reports require proper and deep evaluation of the exact benefits to human health. The aim of the study was to investigate the effect(s) of aqueous extract of *Moringa oleifera* lam leaves on the histo-architecture of male adult Wistar rats' testes. Twelve (12) male adult Wistar rats were used for the study. They were acclimatized and randomly distributed into groups A-D. They were given daily administration of extract of *Moringa oleifera* lam leaves orally for three (3) weeks at doses of 200, 400 and 600 mg/kg were given to groups B, C and D respectively, while group A was given distilled water. Three Wistar rats; one from each group were sacrificed on days 8, 15 and 22. Testes were collected and immediately processed histologically for hematoxylin and eosin stain. The photomicrographs were observed under the microscope with magnifications of x40 and x100. Histological sections of group A revealed that testes section showed normal tissue constituents. For group B, histological sections of testes showed mild seminiferous tubules disruption. For group C, histological sections of testes showed increase seminiferous tubules disruption as well as tubular lumina filled with amorphous eosinophilic materials. Histological sections of testes from group D showed severe seminiferous tubules damages than the other groups. Histological sections of all the treated groups showed that the extract affected the histo-architecture of the testes, thus indicating that the effects were dose-dependent and time-dependent.

Keywords: *Moringa oleifera* lam, Testes, Wistar rats, Male, Histo-architecture

Introduction

Moringa oleifera, Lam, is a natural as well as a cultivated variety of the genus *Moringa* belonging to family *Moringaceae*. *Moringa Oleifera* commonly called Drumstick, Horseradish or Miracle tree. The tree is a fast-growing, drought-resistant tree that is native to the southern foothills of the Himalayas in north-western India. The tree is grown mainly in semi-arid, tropical, and subtropical areas.¹ The plant is grown for food and it is an exceptionally nutritious vegetable tree with varieties of potential value.² The tree is valued mainly for its tender pods, which are esteemed as vegetable, tender leaves and flowers are also used as vegetable. Apart from providing nutrition, it also contributes to the appealing colour, texture, and flavour of the food.³

Moringa oleifera is an important food commodity that has had enormous attention as the 'Natural Nutrition of the Tropics'. The leaves, fruit, flowers and immature pods of this tree are used as a highly

nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa.^{4, 5, 6, 7} It is known as 'Mother's Best Friend' because of its utilisation to increase woman's milk production and is sometimes prescribed for anaemia.^{8, 9}

Although the consumption of different parts of *Moringa oleifera* lam including the roots for various purposes has been widely accepted, Methanolic extracts of *Moringa oleifera* lam roots were found to distort the histo-architecture of both liver and kidneys of guinea pigs.¹⁰ These effects are time-dependent and dose-dependent.

Materials and Methods

Animals and Grouping

Twelve (12) healthy male Albino Wistar rats were allowed to acclimatize to the new environment for two (2) weeks before the administration of the

Moringa oleifera leaf extract. These rats were randomly divided into a group of four (4) with each group containing three (3) Wistar rats and the groups were labelled A, B, C, and D.

Group A which served as the control group was given distilled water, group B was treated with *M. oleifera* leaf extracts of 200 mg/kg body weight daily at the calculated concentration of 10 mg/ml, group C was treated with *M. oleifera* leaf extracts of 400 mg/kg body weight daily at the calculated concentration of 20 mg/ml, and group D was treated with *M. oleifera* leaf extracts of 600 mg/kg body weight daily at the calculated concentration of 30 mg/ml. The grouped animals were placed in two separate plastic cages to avoid stressing the animals.

Preparation of Plant Extract

The leaf powder of *M. oleifera* was measured using weighing balance and macerated. 100 g of the powder was macerated with 1000 ml of distilled water, shaken and allowed to soak completely for 24 hours. The plant-derived aqueous extract tested in this study was prepared and the homogeneous solution was filtered using number 1 Whitman filter paper and the filtrate was placed in a boiling water bath with temperature range of 70°-98°C. The process continued until all the water evaporated and the resulting extracts obtained were crude solid. After the extraction process, the resulting crude solid extracts of *M. oleifera* was dissolved in distilled

water. The extract was stored in sub-zero temperature in the refrigerator.

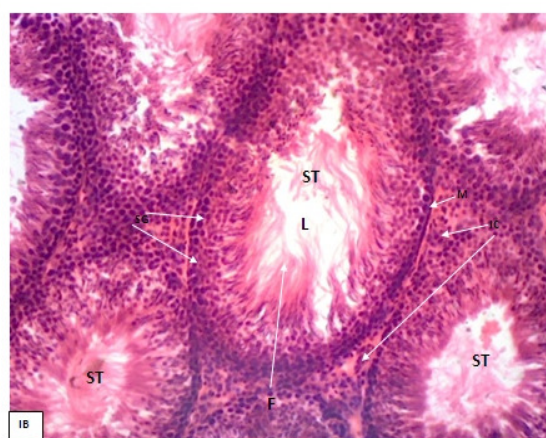
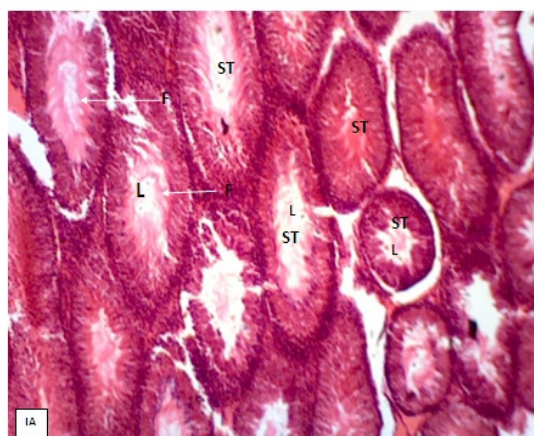
Weight of each Wistar rat was measured with weighing balance before dissolving the extracts in distilled water and the volume administered per rat and concentration of *M. oleifera* leaves were calculated.

Experimental Protocol Doses of 200 mg/kg, 400 mg/kg and 600 mg/kg were administered to groups B, C and D respectively at different concentrations. The extract was administered orally daily in the morning between 8:00 am 10:00 am.

Four rats, one from each group were sacrificed on days 8, 15, and 22 of the experiment by midline incision to expose the ventral abdominal wall. The testes were collected immediately and fixed in a plain transparent container containing 10% normal saline (fixative) for a minimum of 24 hours.

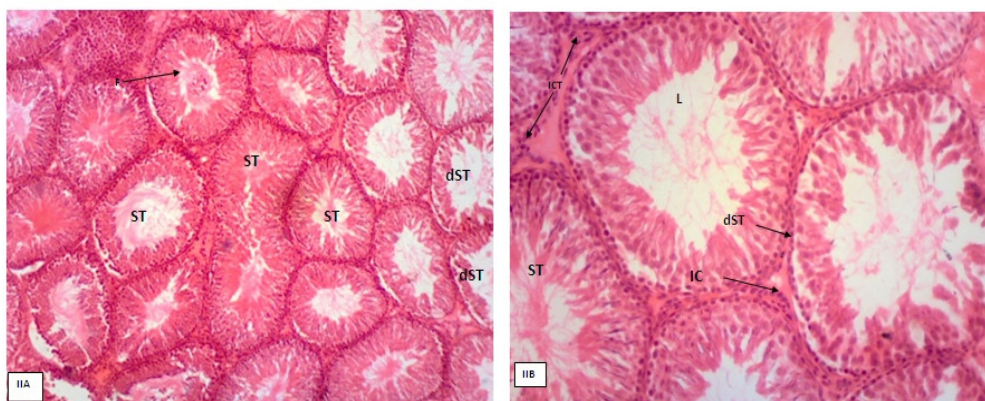
After fixation of the testes in 10% normal saline, the tissue was dehydrated using alcohol of different strengths, then cleared using xylene, then embedded in molten paraffin wax, sectioned and stained using hematoxylin and eosin, placed on glass slide containing albumin as an adhesive, and then covered with coverslip and then mounted on microscope for viewing and snapping.

Results



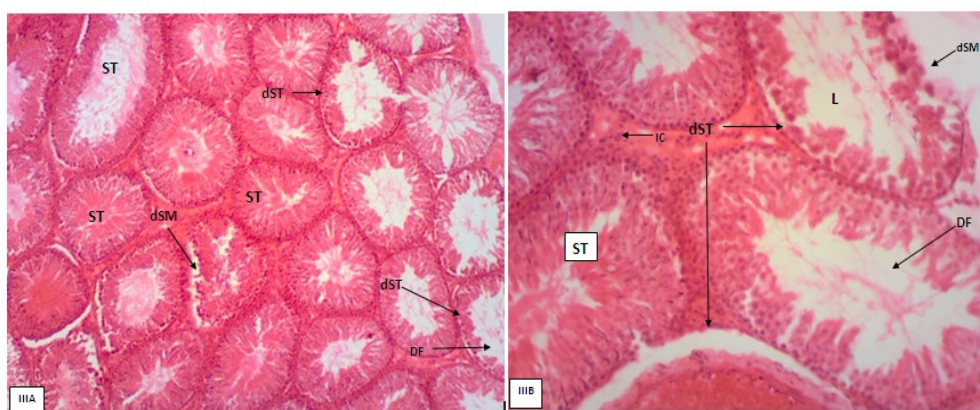
Plates IA and IB: H & E photomicrograph of the testis of adult Wistar rat treated with a distilled water bodyweight of the extracts. The photomicrograph shows the normal histo-architecture of the

seminiferous tubule (ST); normal flagella (F); well-defined spermatogonia (SG); and lumen (L). Magnification x40 (IA), x100 (IB)



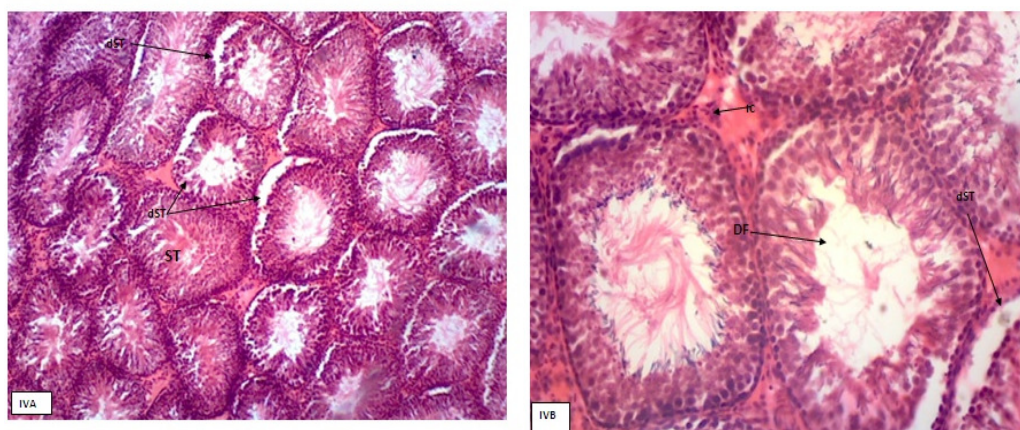
Plates IIA and IIB: H & E photomicrograph of the testis (day 8) of adult Wistar rat treated with 200 mg/kg body weight of the extracts. The histo-architecture shows mild disruption of seminiferous

tubule (dST) and reduced spermatozoa activity; degeneration of spermatogonia layer (SG); and prominent lumen (L). Magnification; x40 (IIA), x100 (IIB).



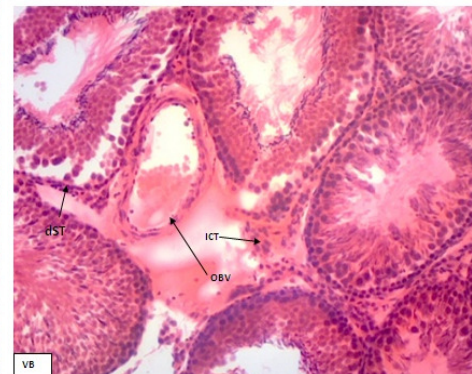
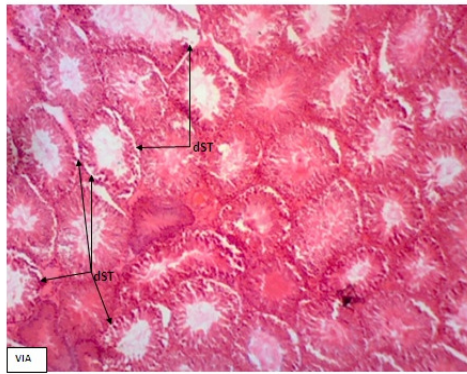
Plates IIIA and IIIB: H & E photomicrograph of the testis (day 8) of adult Wistar rat treated with 400 mg/kg body weight of the extracts. The histo-architecture shows mild disruption of seminiferous

tubule (dST) and reduced spermatozoa activity; mild degenerating seminiferous membrane/epithelium (dSM); prominent lumen (L) and interstitial cell (IC). Magnification; x40 (IIIA), x100 (IIIB)



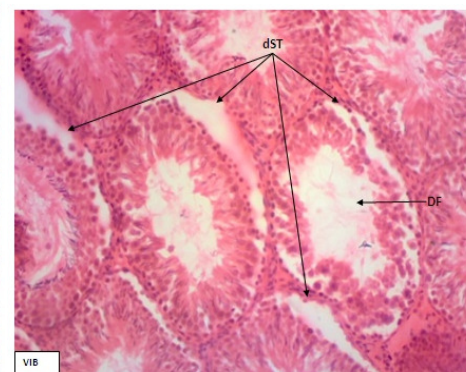
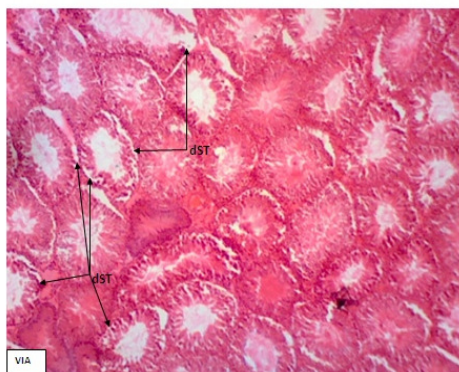
Plates IVA and IVB: H & E photomicrograph of the testis (day 8) of adult Wistar rat treated with 600 mg/kg body weight of the extracts. The histo-architecture shows moderate disruption of

seminiferous tubules (dST) and decrease flagella (DF); mild degenerating seminiferous membrane (dSM); and prominent lumen (L). Magnification: x40 (IVA), x100 (IVB).



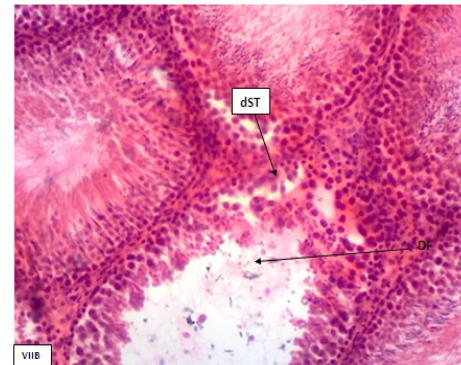
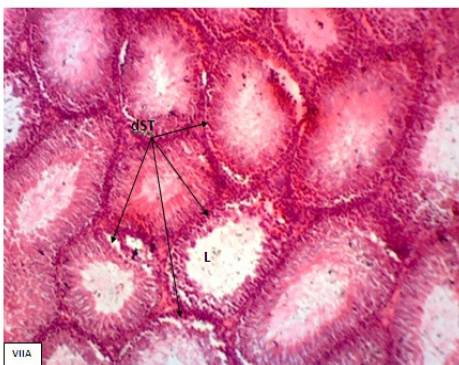
Plates VA and VB: show H & E photomicrograph of the testis (day 15) of adult Wistar rat treated with 200 mg/kg body weight of the extracts. The histo-architecture shows increase disruptions of seminiferous tubules (dST) and decrease flagella

(DF); degenerating seminiferous membrane/epithelium (dSM); and partial occlusion of a blood vessel. Magnification: x40 (VA), x100 (VB).



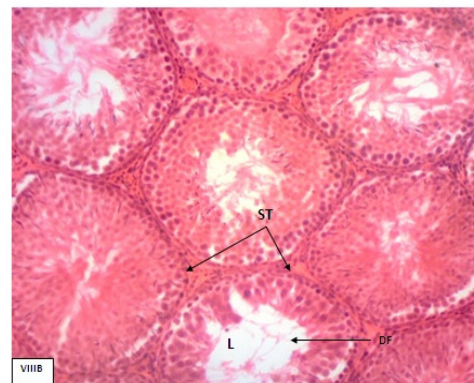
Plates VIA and VIB: H & E photomicrograph of the testis (day 15) of adult Wistar rat treated with 400mg/kg bodyweight of the extracts. The histo-architecture shows severe disruptions of seminiferous tubules (dST) and severe decrease

flagella (DF); degenerating seminiferous membrane/epithelium (dSM); and partial occlusion of the blood vessel. Magnification: x40 (VIA), x100 (VIB).



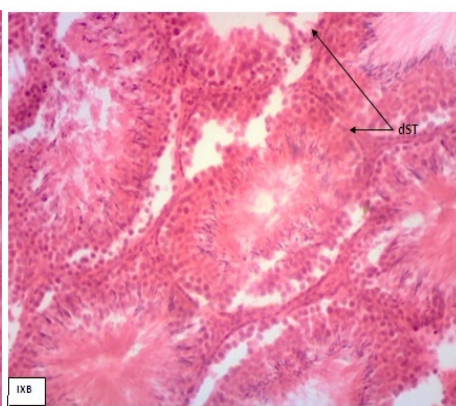
Plates VIIA and VIIB: H & E photomicrograph of the testis (day 15) of adult Wistar rat treated with 600 mg/kg body weight of the extracts. The histo-architecture shows severe disruptions of

seminiferous tubules (dST) and severe decrease/disappearance of flagella (DF); degenerating seminiferous membrane/epithelium (dSM). Magnification: x40 (VIIA), x100 (VIIB).



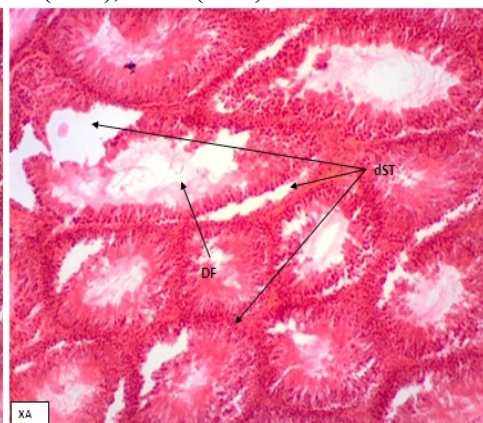
Plates VIIIA and VIIIB: H&E photomicrograph of the testis (day 22) of adult Wistar rat treated with 200 mg/kg body weight of the extracts. The histo-architecture shows Tunica albuginea (TA); mild

disruption of seminiferous tubule (dST) and reduced spermatozoa activity and lumen (L). Magnification; x40 (VIIIA), x100 (VIIIB).



Plates IXA and IXB: H&E photomicrograph of the testis (day 22) of adult Wistar rat treated with 400 mg/kg body weight of the extracts. The histo-architecture shows severe disruptions of

seminiferous tubules (dST) and severe decrease flagella (DF); degenerating seminiferous membrane/epithelium (dSM). Magnification: x40 (IXA), x100 (IXB).



Plates XA and XB: H&E photomicrograph of the testis (day 22) of adult Wistar rat treated with 600mg/kg body weight of the extracts. The histo-architecture shows severe disruptions of seminiferous tubules (dST); decrease/disappearance of flagella (DF); degenerating seminiferous membrane/epithelium (dSM); Magnification: x40 (XA), x100 (XB).

Discussion

The testicular tissues of the experimental animals such as the seminiferous tubules (ST) were well defined and the interstitial tissues also defined. The seminiferous tubule epithelium (STE) was well defined, normal flagella (F), well-defined spermatogonia (SG), spermatids, spermatozoa and has a normal histo-architecture; so also the central lumen (L). However, mild disruption of seminiferous tubule (dST) and reduced spermatozoa activity; degeneration of spermatogonia layer (SG); and prominent lumen (L); moderate disruption of seminiferous tubules (dST) and decrease flagella (DF); mild degenerating seminiferous membrane (dSM); and prominent lumen (L). With longer time of administration of the extract, histopathological finding showed severe disruptions of seminiferous tubules (dST) and severe decrease flagella (DF); degenerating seminiferous membrane/epithelium (dSM) also become evident.

The experimental finding is in line with finding of ¹¹ who reported an extensive disruption of the histo-architecture that was observable at all magnifications. The seminiferous tubules had grossly disrupted epithelium and consequently, large lumens. At the higher magnifications, it is evident that the disruptions cut across the various zones or layers of germinal cells whose arrangements also corresponded with the stages of development. Consequently, spermatogenesis would be generally compromised irrespective of the stage of the maturation of the cells. It was evident that *M. oleifera* leaf extract was found to affect the normal histology of the testicular tissue where spermatozoa are produced which may affect fertility depending on the degree of the effect ¹⁰ had earlier reported the deleterious effects of the root extract on the testis. Traditional and early experimental reports have implicated that, this plant product has fertility influencing effects such as being abortifacient ^{12, 13}, and as a birth control effect ^{14, 15, 16, 17, 18}.

Conclusion

The study showed that aqueous extract of *Moringa oleifera lam* leaves distorted the histo-architecture of testes of male adult Wistar rats. Thus, *Moringa oleifera* leaf extract was found to affect the normal histology of the testicular tissue where spermatozoa are produced which may affect fertility depending

on the degree of the effects which were found to be time-dependent and dose-dependent respectively.

Conflict of interest

Declared none.

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