JCHR (2023) 13(3), 565-575

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Subchronic Toxicological Studies of Methanol and n-Hexane Extracts of *Leptadenia hastata* (pers) Decne Leaves Used as Antihypertensive Agent

Hadiza Lami Muhammad[*1], Rahinat Garba[1], Abubakar Siddique Abdullah[2], Hussaini Anthony Makun[1], Musa Bola Busari[3], Hadiza Kudu Muhammad[1], Funmilola Sherifat Adefolalu[4]

[1]*Africa Centre of Excellence for Mycotoxin and Food Safety, Federal University of Technology, Minna, Nigeria*

[2]*Department of Internal Medicine, Ahmadu Bello University Zaria, Nigeria*

[3]*Centre for Genetic Engineering and Biotechnology, Global Institute for Bioexploration Unit, Federal University of Technology, Minna, Nigeria*

[4]*Malaria Research Unit, Department of Biochemistry, Federal University of Technology, Minna, Nigeria*

(Received: 19 June 2021 Accepted: 7 January 2023)

KEYWORDS

Toxicity;
Extract;
Phytochemical

ABSTRACT: *Leptadenia hastata* leaf extracts are used in the folkloric treatment of hypertension and its attendant complications. Sub-chronic toxicological study of the methanol and n-hexane leaf extracts of *L. hastata* was carried out orally on Swiss albino rats for 28 days. Doses of 100, 300, and 600 mg kg^{-1} bodyweights of both extracts were administered through the oral route once daily to the rats in respectively labeled test groups while the control group with normal saline (0.5 ml). *L. hastata* methanol extract showed a moderate presence of alkaloids (0.92±0.14) and cardiac glycosides. The LD_{50} of both extracts is >5000 mg kg^{-1} bodyweight. ALT, AST, ALP, and total protein were all significantly high in 600 mg kg^{-1} bodyweight of the extract (n-hexane)-treated group by a 2% reduction in bodyweight on the 12th day. Lipids in both extract-treated groups were reduced with a concomitant increase in HDL of the methanol extract-treated groups and a decrease in the extract (n-hexane)-treated groups. PCV and RBC significantly increased ($p<0.05$) and decreased ($p>0.05$) in the methanol and n-hexane extract-treated groups respectively, while the WBC significantly increased in the extract (n-hexane)-treated groups. Only 600 mg kg^{-1} bodyweight of the extract (n-hexane)-treated group showed a decrease in liver and kidney weights with an increase in the weight of the heart. Electrolytes were significantly reduced in 600 mg kg^{-1} bodyweight of the extract (n-hexane)-treated group while urea, creatinine, direct, and total bilirubin increased in the extract (n-hexane)-treated groups. *L. hastata* extracts at 600 mg kg^{-1} bodyweight may be toxic.

INTRODUCTION

About 80 % of African and Asian countries depend on plants as the main food source and use them as medicine for primary health needs. The rural communities use plants either as decoctions, packaged products in powdered form or the leaves are made into slurry, cooked as soups, or eaten raw to treat pathogens. Whether taken as food or medicine, there is the general belief that all plant products are safe being from the natural source and hence, the indiscriminate use [1]. The World Health Organization WHO [2] has however observed that inappropriate use of plants as therapeutic agents can harm consumers most probably because the

*Corresponding author: hadizalami@futminna.edu.ng (H. Lami Muhammad)
DOI: 10.22034/jchr.2023.1933560.1338

doses are never regulated. Preclinical therapeutic trials backed by several research models suggested that extracts from plant hold numerous properties; pharmacological and nutritional but adequate information on the toxicological profile of most medicinal plants is grossly inadequate. According to WHO, one quarter of conventional medications used in the United States are derivative from plant origin [3]. Today in modern medicine, 80% of the active phytocompounds presently isolated from complex plants demonstrates a positive relationship between their use in modern therapeutic medicine, and in traditional medicine of the plants from which they are derived [4]. Any plant part which could be utilized for therapeutic/curative purposes or serve as leads for the synthesis of valuable drugs due to the presence of certain substances is termed medicinal [5]. In Nigeria, indigenous medicinal plants have contributed to the primary health care system of the rural populace. There is however inadequate scientific authentication of the use of most plants as drug agents as claimed by the folkloric use and inadequate data on their toxicological/safe profile which is much required. The secondary metabolites that are responsible for the color and organoleptic properties in plants are also accountable for most of their pharmacological/medicinal properties [6]. These secondary metabolites have biological significances that are already established as plants' protectors against predators.

Leptadenia hastata (pers) Decne (family Asclepiadaceae) is a non-domesticated edible vegetable widely used in tropical Africa. It grows on drylands in sandy soil with crawling latex stems, glabrescent leaves, glomerulus, racemes flowers, and follicle fruits [7]. Ethnobotanical information obtained from herbal medicine practitioners in the Northern part of Nigeria made it known that the leaves of *L. hastat*a are boiled and the infusion is taken orally to treat diarrhea and hypertension without cognizance of overdose. In a report by Soni *et al*. [8], children are given the infusion to cure stomachache, while other local herbalists use the leaves from the plant to manage high blood pressure, and treat catarrh, and certain skin diseases. In Senegal, sap or root decoction is used for ophthalmic infections, and in association with other plants, *L. hastata* leaf decoction is given to suckling babies with gastroenteritis [9]. In Burkina Faso, *L. hastata* leaves are chewed for sexual potency, leaf decoction as anti-trypanosomiasis, and applied onto the skin to treat rashes [10]. The antimicrobial properties of *L. hastata* have been reported [11]. The World Health Organization regarding the selection of herbal medicine for use in health care, has emphasized that safety should be a superseding criterion. Since *L. hastata* is claimed in folklore medicine as a cure for infections and to manage some disorders (without recourse to its safety), it is necessary to evaluate its toxicological profile to scientifically validate the claim and establish the safe dose for clinical applications especially as it is always used over a long period.

MATERIALS AND METHODS

Reagents and Chemicals

All the reagents and chemicals used were of analytical grade and products of Sigma Chemical Co. USA. They include methanol, n-hexane, dimethyl sulfoxide, normal saline, and distilled water.

Apparatus and Equipment

The apparatus used for the research include test tubes, beakers, conical flasks, burettes, round bottom flasks, measuring cylinders, masking tape, volumetric flasks, separating funnels, Whatman no.1 filter paper, electronic weighing balance, drying oven, rotatory evaporator, water bath, foil, mortar and pestle, and electronic blending machines. All glass wares were carefully washed and rinsed with liquid detergent and distilled water, then oven-dried at 105°C.

Sample Collection and Preparation

Fresh leaves of *Leptadenia hastata* were collected from the Maikunkele district in Minna, Niger State, Nigeria. They were identified morphologically at the Plant Biology Department of the Federal University of Technology, Minna, Niger State, Nigeria. Fresh leaves of *L. hastata* were destalked, washed with clean water, and allowed to dry at room temperature in the departmental laboratory for two weeks. The dried leaves were milled with an electric blender (EUROSONIC, ES-242) to obtain a smooth powder and stored till ready for use.

Extraction of Plant Material

Extraction with Methanol

A thousand (1000) ml of methanol was added to two hundred grams (200 g) of powdered *Leptadenia hastata* leaves and exhaustedly extracted for 72 hours at 65^{o}C. The resultant methanol extract was concentrated in a water bath at 35^{o}C for complete solvent evaporation. The dried extract was stored in a refrigerator at -4^{o}C till required for use.

Extraction with n-Hexane

A thousand (1000) ml of n-hexane and distilled water in a ratio of 5:1 was added to two hundred grams (200 g) of powdered *Leptadenia hastata* leaves and exhaustedly extracted for 72 hours at 50^{o}C. The resultant extract was concentrated in a water bath at 35^{o}C for complete solvent evaporation. The semi-dried extract was stored in a refrigerator at -4^{o}C till required for use.

Qualitative Screening for Secondary Metabolites

The extracts were screened for secondary metabolites according to standard methods as described by Sofowora [12].

Quantitative Determination of Secondary Metabolites

The extracts were screened for quantitative secondary metabolites in accordance with standard methods as described by Chadva *et al*. [13] for flavonoids, Singh *et al*. [14] for phenols, Harbourne, [15] for alkaloids and tannins, and Olaniyan *et al*. [16] for saponins.

Experimental Animals

Swiss albino rats weighing between (117-159) g were purchased from the animal breeding unit of the Department of Physiology, University of Ibadan, Oyo State, Nigeria. They were housed in plastic cages with wood shavings as beds, maintained under standard laboratory conditions of 37^{o}C, and allowed unrestricted access to grower marsh as feed *ad libitum* as they adapt for two (2) weeks, falling in line with globally accepted ethics for human and laboratory experimental animals outlined in the Canadian Council of Animal Care guidelines and Protocol Review [17] as approved by the ethical committee of Federal University of Technology Minna, Niger State.

Acute Oral Toxicity Test

The acute oral toxicity test was carried as described by Lorke, [18]. Normal saline and dimethyl sulfoxide (DMSO) were used as vehicles for the methanol and n-hexane extracts respectively.

Animal Grouping

Animals were grouped into seven, each comprising three rats:

Group 1: received 100 mg kg^{-1} bodyweight of methanol extract

Group 2: received 300 mg kg^{-1} bodyweight of methanol extract

Group 3: received 600 mg kg^{-1} bodyweight of methanol extract

Group 4: received 100 mg kg^{-1} bodyweight of n-hexane extract

Group 5: received 300 mg kg^{-1} bodyweight of n-hexane extract

Group 6: received 600 mg kg^{-1} bodyweight of n-hexane extract

Group 7: received 0.5 ml of normal saline as a control.

Administration of Extracts

Animals in their respective groups received oral doses of extracts as indicated in 2.7 above. One (1) g of the methanol extract was dissolved in 10 ml of distilled water while 1 g of the n-hexane extract was dissolved in 10 ml of dimethyl sulfoxide (DSMO).

Termination of the Experiment

Extract administration was for a period of 28 days while on the 29th day, the experiment was ended and the animals were euthanized after been anesthetized under chloroform vapor. The blood samples were collected into sample bottles for biochemical and hematological parameters via carotid puncture. The heart, kidneys, and liver, were taken out from dissected rats, instantly cleaned using physiological saline, and weighed.

Calculation of Organ/Bodyweight Ratio

The organ/bodyweight ratio was calculated using the formular;

$$\frac{\text{weight (g) of each organ}}{\text{weight (g) of the rat before sacrifice}}$$

Preparation of Serum

The non-heparinized blood samples were and centrifuged at 1000 rpm for 5 minutes after allowing it clot using a centrifuge (Hettich Universal). The supernatant was collected and used for the evaluation of biochemical parameters.

Serum Biochemical Investigations

Determination of Serum Lipid Profiles

The serum total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined by using Randox diagnostic kits according to the methods described by the maker.

Determination of Total Protein

Total protein was determined by using a commercial kit (AGAPE, Switzerland) as described in the manual by the manufacturer.

Determination of Enzyme Activities

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in the serum were determined using Randox diagnostic kits as described in the manual by the manufacturer.

Determination of Haematological Parameters

Total red blood cells (RBC), and white blood cells (WBC), were determined using the Swelab auto hematology Analyzer while the packed cell volume (% PCV) was read directly from the hematocrit reader.

Statistical analysis

The Statistical Product for Social Solution (SPSS version 16.0) software was utilized in analysing all the data obtained, results were expressed as Mean ± Standard error mean (SEM) using one-way analysis of variance (ANOVA), followed by Turkey, Duncan, and Dunnett's multiple comparison tests. The values of $p<0.05$ were said to be statistically significant.

RESULTS

Qualitative Phytochemical Constituents of Leptadenia hastata

Only alkaloids and cardiac glycosides are moderately present (Table 1) in the methanol leaf extract of *Leptadenia hastata.*

Quantitative Phytochemical Constituents of Leptadenia hastata

Alkaloids, saponins, tannins, phenols, and flavonoids were present in the methanol extract in descending order as shown in Table 2.

Acute Toxicity Test of Leptadenia hastata

Signs such as alertness, breathlessness, coma, convulsion, diarrhea, motor activity, restlessness, and tremor were not observed at various doses of extracts. The rats were active physically with no death was noted at dose of up to 5000 mg kg^{-1} bodyweight as presented in Table 3.

Effect of *Leptadenia hastata on Bodyweight of Experimental Rats*

The extracts had no significant effect on animal weights (Figure 1) except on the 8^{th} day when the 300 mg kg^{-1} bodyweight methanol extract-treated group showed a significant reduction in bodyweight.

Effect of *Leptadenia hastata on Liver Function Parameters of Experimental Rats*

The ALT and AST activities increased and decreased respectively in all groups (Figure 2) except the 600 mg kg^{-1} bodyweight methanol extract-treated group which showed no significant difference in AST with the

reference (control) group. Only the 100 mg kg^{-1} bodyweight methanol extract-treated group showed no significant difference in total protein (TP), other groups demonstrated a significant increase in total protein.

Effect of *Leptadenia hastata on Some Haematological Parameters of Experimental Rats*

All methanol extract-treated groups showed a significant increase in PCV (%) in contrast to the n-hexane extract-treated groups that gave a significant decrease in PCV (%) levels in a dose-dependent manner. The WBC of n-hexane extract-treated groups also increased significantly as shown in Figure 3.

Effect of *Leptadenia hastata on Relative Organ Weight of Experimental Rats*

Except for the 100 mg kg^{-1} bodyweight of the methanol extract-treated group, all other groups demonstrated a significant increase in the weight of the liver with the 600 mg kg^{-1} bodyweight of the n-hexane extract-treated group showing up to 400% increase. The extracts however demonstrated no effect on the weight of the heart as depicted in Figure 4.

Effect of *Leptadenia hastata on Urea and Creatinine of Experimental Rats*

In Figure 5, all the n-hexane extract-treated groups showed significant ($p<0.05$) elevation in urea and creatinine.

Effect of *Leptadenia hastata on Direct and Total Bilirubin of Experimental Rats*

In Figure 6, all the n-hexane extract-treated groups showed significant ($p<0.05$) elevation in both direct and total bilirubin.

Effect of *Leptadenia hastata on Serum Electrolytes of Experimental Rats*

All n-hexane extract-treated groups exhibited a significant decrease in Cl^- as well as a non-significant increase in Na^+, K^+, and HCO_3^- as seen in Figure 7.

Effect of *Leptadenia hastata on Serum Lipids of Experimental Rats*

In Figure 8, the 300 and 600 mg kg^{-1} bodyweight n-hexane extract-treated groups demonstrated no difference in CHOL and LDL levels. The extracts were able to reduce the TAG and elevated the HDL.

Table 1. Qualitative Secondary Metabolites in *Leptadenia hastata* Leaf Extracts.

Secondary metabolite	n-Hexane Extract	Methanol Extract
Alkaloids	-	++
Phlabotannins	-	+
Steroids	-	-
Cardiac glycosides	-	++
Anthraquinones	-	-
Terpenes	-	-
Flavonoids	+	+
Phenols	+	+
Tannins	-	+
Saponins	-	+

Key: + =mildly present; ++ =moderately present;- = absent

Table 2. Quantitative Secondary Metabolites in *Leptadenia hastata* Leaf Extracts.

Secondary metabolite (mg/g)	n-Hexane Extract	Methanol Extract
Alkaloids	0.007±0.00	0.92±0.14
Flavonoids	0.21±0.01	0.52±0.02
Phenols	0.46±0.05	0.62±0.03
Tannins	0.19±0.01	0.82±0.06
Saponins	0.04±0.00	0.83±0.05

Table 3. Acute Oral Toxicity Test of Methanol and n-hexane Leaf Extracts of *Leptadenia hastata*

Phase 1		Phase 2	
Dose (mg kg^{-1} bodyweight)	**Mortality**	**Dose (mg kg^{-1} bodyweight)**	**Mortality**
10	0/3	1600	0/3
100	0/3	2900	0/3
1000	0/3	5000	0/3

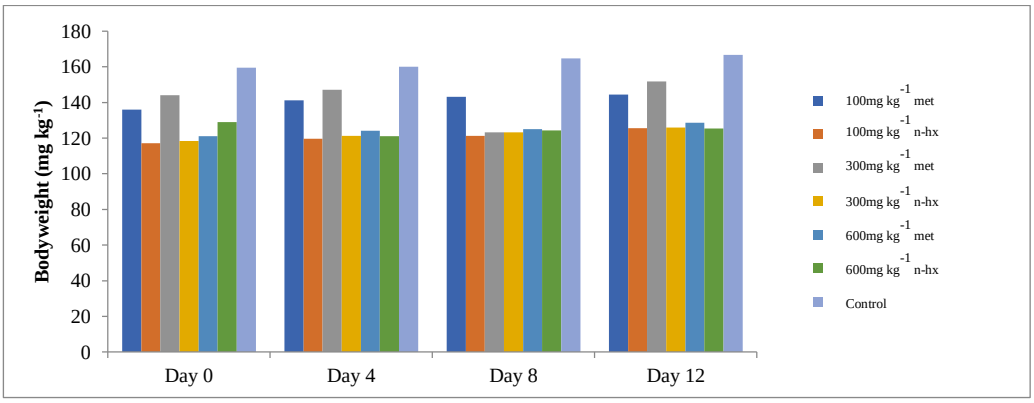

Figure 1. Effect of Various Doses of Extracts on BodyweightKey: met-methanol extract, n-hx-n-hexane extract.

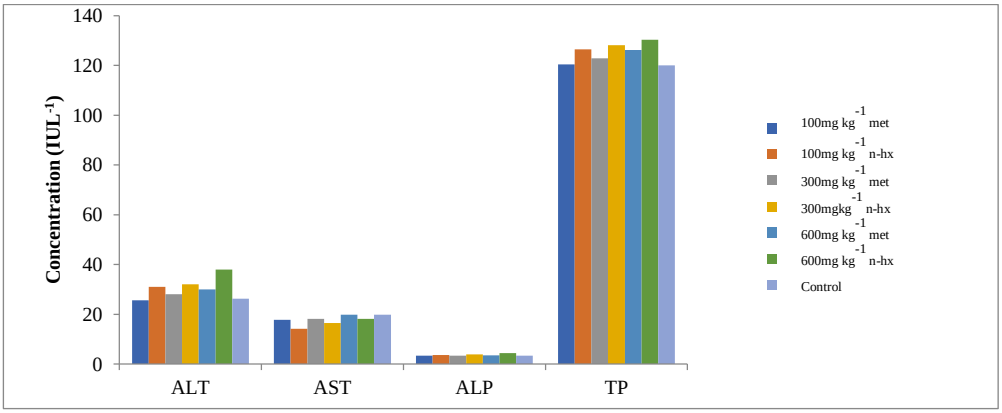

Figure 2. Effect of various doses of extracts on enzyme activities and total protein.
Key: met-methanol extract, n-hx-n-hexane extract, ALT-alanine aminotransferase, AST-aspartate aminotransferase, ALP-alkaline phosphatase, TP-total protein.

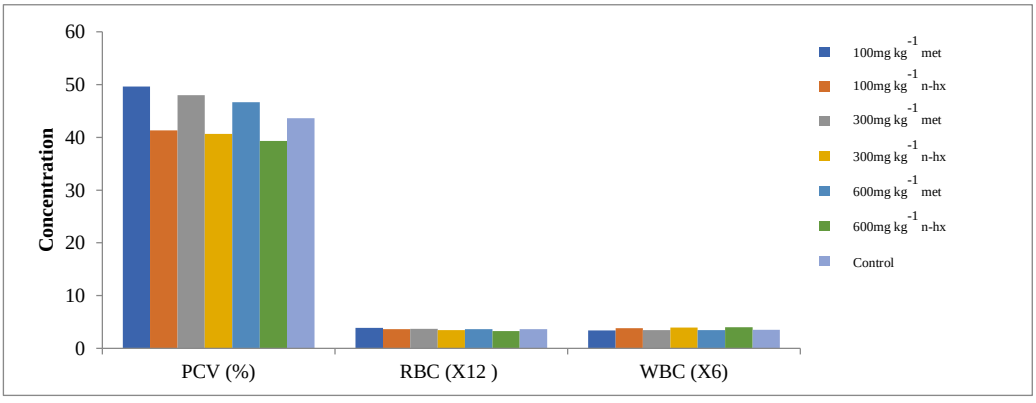

Figure 3. Effect of Various Doses of Extracts on Some Haematological Parameters.
Key: met-methanol extract, n-hx-n-hexane extract, PCV-packed cell volume, RBC-red blood cell, WBC-white blood cell.

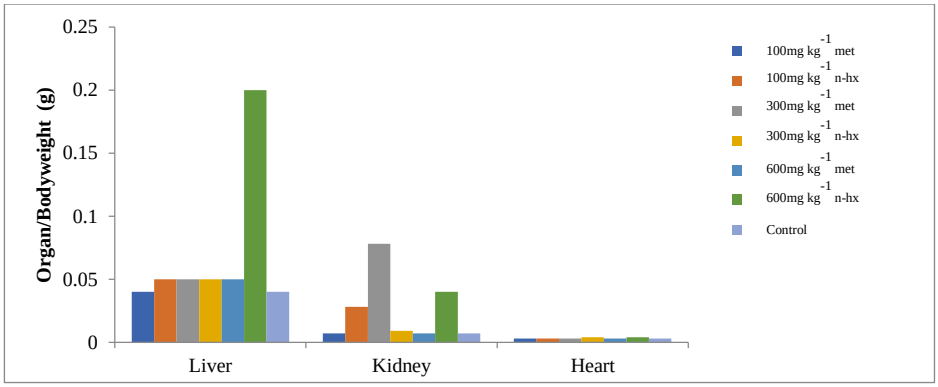

Figure 4. Effect of Various Doses of Extracts on Relative Organ Weight. Key: met-methanol extract, n-hx-n-hexane extract.

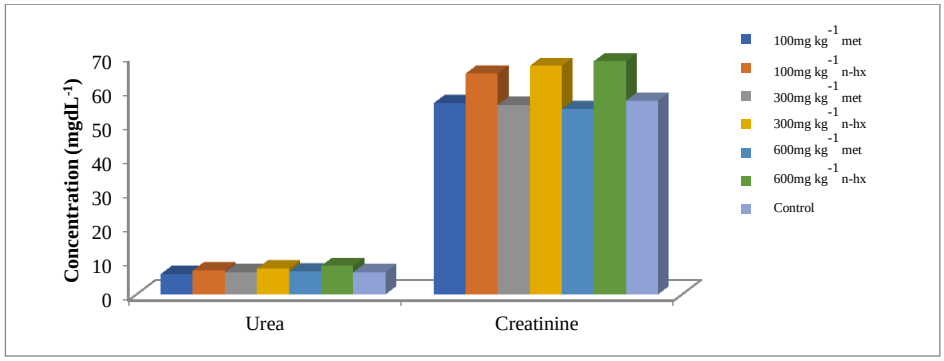

Figure 5. Effect of Various Doses of Extracts on Urea and Creatinine. Key: met-methanol extract, n-hx-n-hexane extract.

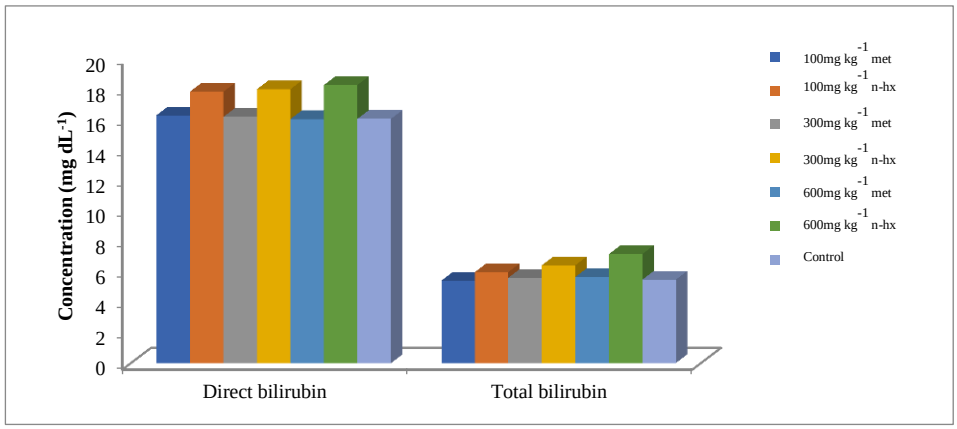

Figure 6. Effect of Various Doses of Extracts on Direct and Total Bilirubin. Key: met-methanol extract, n-hx-n-hexane extract.

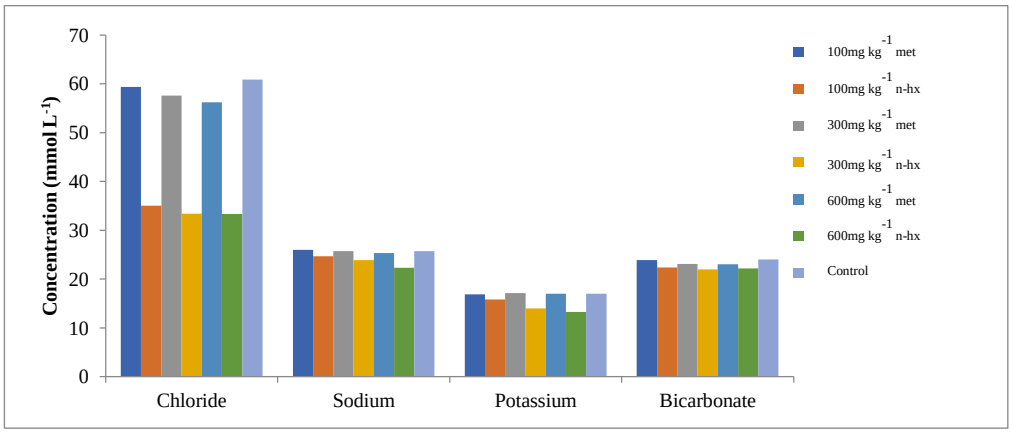

Figure 7. Effect of Various Doses of Extracts on Serum Electrolytes. Key: met-methanol extract, n-hx-n-hexane extract.

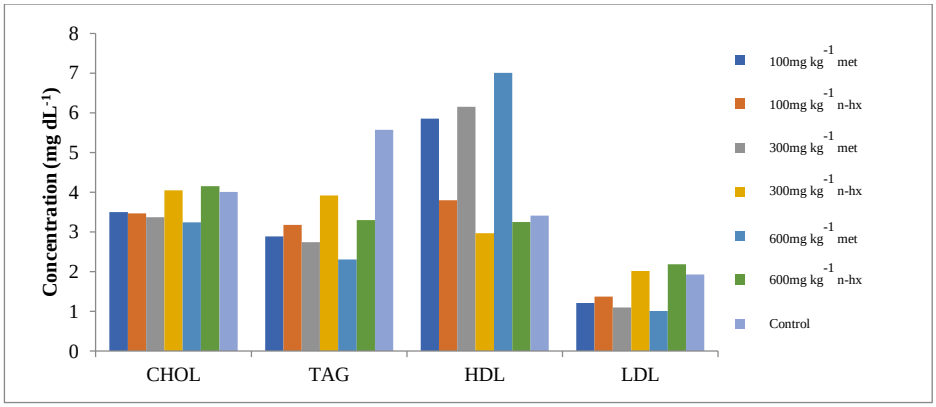

Figure 8. Effect of Various Doses of Extracts on Serum Lipids
Key: met-methanol extract, n-hx-n-hexane extract, CHOL-total cholesterol, TAG-Triglyceride, HDL-high density lipoprotein, LDL-low density lipoprotein.

DISCUSSION

Phytochemicals elicit their pharmacological potentials by interfering with certain metabolic processes, modulating gene expression, and signaling transduction pathways or inhibiting the growth of microorganisms. They can be used as chemopreventive or chemotherapeutic agents since the molecular mechanisms are common to both. Alkaloids were found substantially (0.92±0.14) (Tables 1 & 2) in the methanol extract and have been reported to be effective against trypanosomes [19], and plasmodia [20]. The medicinal potential of saponins against eukaryotic cells is linked with membrane permeability property as it complexes with cholesterol [21]. Tannins are described to either bring cellular activities to a standstill or cause death in *Staphylococcus aureus*, also, certain microbial enzymes in raw culture filtrates/purified forms when mixed with tannins, are inhibited. Saponins and tannins have been found in methanol extract in substantial concentrations (0.83 ± 0.05) and (0.82 ± 0.06) respectively (Tables 1 & 2).

Experimental animals in their respective groups were administered various doses of the two extracts according to Lorke's method to get the median lethal dose (LD_{50}) on which to base the toxicity test. All animals in the two extract-treated groups tolerated the extracts with no sign of toxicity such as breathlessness, coma, convulsion, diarrhea, motor activity, restlessness, and tremor. The rats were active physically and no death was noted at the dose of up to 5000 mg kg^{-1} bodyweight twenty-four hours post-administration of the extracts. The safe doses of these extracts were therefore found to be >5000 mg kg^{-1} bodyweight (Table 3). According to the Guidance Document Acute Toxicity Test based on oral LD_{50} value which was recommended by [22], the methanol and n-hexane extracts of *L. hastata* may be allotted as class 5 (LD_{50}>2000 mg kg^{-1} bodyweight), which was referred to as the class with lowest toxicity.

Impairment in the normal function of organs may be a result of an alteration in the overall bodyweight or organ: bodyweight ratio [23]. The extract doses did not affect the body weight of the animals (Figure 1) because, at the start of the experiment, the control group had a higher weight than the extract-treated groups.

The quantification of enzyme activities in body fluids and tissues plays a significant role in investigating and diagnosing disease, as well as in cellular and tissue damage [24]. The activity of alanine aminotransferase (ALT) increased significantly (p>0.05) in a dose-dependent manner in the two extract-treated groups (except the 100 mg kg^{-1} bodyweight of the methanol extract) (Figure 2). ALT is considered a more specific and sensitive indicator of hepatocellular damage. Increased serum ALT may be due to hepatocellular necrosis, regenerative/reparative activity, hepatic microsomal induction, or even extrahepatic factors like a muscle injury. There is therefore no conclusive remark regarding the effect of the methanol and n-hexane extracts of *Leptadenia hastata* until further histopathological studies of the liver are carried out. The decrease in the activity of aspartate aminotransferase (AST) observed in Figure 2 may be a result of its decreased hepatocellular production/release, inhibition, and/or reduction of enzyme activity and interferences such as the effect of PH, Temperature, and Substrate Saturation with reduces the enzyme's reaction rates.

Wallace, [25] hypothesised that since the liver is a major organ for the synthesis of protein, any damage to hepatocytes can lead to alteration in its production capacity. As with ALT, serum total protein increased in all groups (except the 100 mg kg^{-1} bodyweight of the methanol extract). This increase may be resulting from increased synthetic function of the liver, impaired hepatocellular function, or dehydration. An increase in alkaline phosphatase (ALP) activity may be due to an increase in its synthesis coupled with increased biliary pressure [26]. The two extract-treated groups however elicited no increase in the activity of ALP as observed in Figure 2.

The hemopoietic system is amid the most subtle targets for toxins, and also an important index to ascertain the physiological and pathological status of humans [27]. Its complications entail abnormalities relating to structure, function, and metabolism of RBC, WBC, and platelets [28]. The chief goal for evaluating RBC is to evaluate anaemia and as well as normal hematopoiesis [29]. Packed cell volume (PCV) signifies the volume of RBC in 100 ml of blood and aids to determine the one's hydration state, extent of anemia and diagnose polycythemia [30]. All methanol extract-treated groups showed a dose-dependent appreciable increase in % PCV (Figure 3) leading to an increase in erythropoiesis in contrast to the n-hexane extract-treated groups. It may be possible that the methanol (a polar solvent) was able to extract the hematinics in the leaves of *L. hastata.* The two extracts at various doses did not exhibit any differences in RBC when compared to the control group. The white blood cell (WBC) count measures the total number of white blood cells in body available at that time, that shield the blood against opportunistic infection. WBC count increased in all the n-hexane, extract-treated groups. This is in line with the study carried out by [31].

Doses of the two extracts elicited a significant increase in the weight of the liver (except for 100 mg kg^{-1} bodyweight of the methanol extract) (Figure 4). The highest weight increase was particularly observed in the 600 mg kg^{-1} bodyweight of the n-hexane extract. As with the liver, the weight of the kidney increased significantly in all groups (except 100 mg kg^{-1} bodyweight of the methanol extract) with the highest increase in weight found in the 300 mg kg^{-1} bodyweight of the methanol extract group. The extracts did not affect the weight of the heart.

Estimation of serum creatinine alongside urea and electrolytes are used as investigative tests to assess kidney function. The kidneys function in controlling urea elimination and electrolytes reabsorption in the bloodstream. These processes occur in the glomeruli and renal tubules respectively [32]. Consequent to normal glomerular function compromise, wastes typically emptied by the kidneys such as creatinine and urea gather in the body fluid. Urea is a nitrogenous (non-protein) substance that gathers in the plasma when renal excretion is low, elevation of blood urea (uremia) due to excess protein in the diet, dehydration, intestinal bleeding (hemorrhage), and shock. A non-significant elevation in serum urea was observed in 600 mg kg^{-1} bodyweight of the n-hexane extract-treated group (Figure 5), and this aligns with the findings of [33] that investigated the acute and sub-chronic toxicity studies of kernel extracts of *Sclerocarya birrea* in rats. A significant increase ($p<0.05$) in creatinine was also observed in all the doses of n-hexane extract. This significant increase suggests that the usual elimination of this biomolecule by the kidney was disrupted.

Bilirubin is lipophilic and transported in the plasma in a conjugated (non-covalent) form to albumin. The bilirubin-albumin complex goes into the liver and where it dissociates and the bilirubin is taken up by the sinusoidal surface of the liver cells via active transport (carrier-mediated). In the liver, the bilirubin is conjugated with two molecules of glucuronic acid, and almost all the bilirubin (>98%) that enters the bile is in the conjugated form. All the doses of n-hexane extract exhibited a significant increase ($p<0.05$) in direct bilirubin while only the 600 mg kg^{-1} bodyweight of the same extract showed a similar increase in total bilirubin (Figure 6). These finding corroborate with the report by [34] which confirmed that the increase in plasma bilirubin (hyper-bilirubinaemia) may result from the decrease in its uptake by the liver, in its conjugation, or increase in its production from hemolysis.

Chloride in collaboration with other electrolytes helps to control the amount of fluid in the body and as well as

maintain the acid-base balance. The elevation in Cl^- may be due to impairment of renal function.

CONCLUSIONS

Leptedenia hastata, though used in folkloric medicine for the treatment of hypertension, could be unsafe at high doses. Thus, caution should be taken during its usage for medicinal purposes particularly, at such doses.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Africa Centre for Excellence for Mycotoxin and Food Safety and Centre for Genetic Engineering and Biotechnology, Global Institute for Bio exploration Staff for giving them access to their Laboratories.

Funding

This research has not received funding from any source.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Bandaranayake W.M., 2006. Quality control, screening, toxicity, and regulation of herbal drugs. In: Modern Phytomedicine: Turning Medicinal Plants into Drugs, Ahmad I., Aqil F., Owais, M., Eds., Weinheim: Wiley-VCH GmbH & Co. KGaA. pp. 25–57.

2. World Health Organization (WHO), 2008. WHO media center fact sheet. http://www. who.int/ mediacenter/fact sheets/fs134/600. (Assessed May, 14th 2009).

3. Muhammad H.L., Makun H.A., Kabiru A.Y., Mann A., Busari M.B., Abdullah A.S., Fatima A., 2015. *In vitro* antibacterial activity and *in vivo* acute toxicological studies of *Nelsonia campestris* aqueous leaf extract. International Journal of Biochemistry Research and Review. 7(1), 27-35.

4. Boussahel S., Dahamna S., Ruberto G., Siracusa L., Harzallah D., 2013. Phytochemical study and antioxidant activities of leaf extracts from *Rhamnus alaternus* L. Pharmacognosy Communications. 3(1), 46-53.

5. World Health Organization (WHO), 2010. Department of Non-communicable Disease Surveillance. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. http://whqlibdoc.WHO International. (Assessed July 25th, 2011)

6. Sahoo N., Manchikanti P., 2012. Herbal drug regulation and commercialization: An Indian Industry Perspective. Journal of Alternative and Complementary Medicine. 19, 957-963.

7. Umaru I.J., Ahmed B.F., Umaru A.H., Umaru I.K., 2018. Leptadenia Hastata (pers) Decne: Phytochemical, pharmacological, biotechnological, botanical, traditional uses and agronomical aspects. European Journal of Pharmaceutical and Medical Research. 5(6), 109-119.

8. Soni P., Siddiqui A.A., Dwivedi J., Soni V., 2012. Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: An overview. Asian Pacific Journal of Tropical Biomedicine. 2(12), 1002-1008.

9. Akanji M.A, Salau A.K., Yakubu M.T., 2013. Safety evaluation of aqueous extract of *Crateva adansonii* leaves on selected tissues of rats. Fountain Journal of Natural and Applied Sciences. 2(1), 17-28.

10. Das N., Goshwami D., Hasan S., Raihan S.Z., 2015. Evaluation of acute and sub-acute toxicity induced by methanol extract of *Terminalia citrine* leaves in Sprague dawley rats. Journal of Acute Diseases. 4(4), 316-21.

11. Olaoluwa T.A., Odutola O., Olugbenga O.A., Funmilayo D.O., Sunday O.O., Afolayan A.J., 2015. Alkaline phosphatase, aspartate aminotranferase, and alanine aminotransferase activities in selected tissues of rats fed on processed atlantic horse mackerel (*Trachurus trachurus*). Advances in Bioscience and Biotechnology. 73, 5942-5948.

12. Sofowara A.E., 1993. Medicinal plant and traditional medicine in Africa. Volume 2. Spectra Books Ltd: Nigeria. pp. 150-156.

13. Chavda R., Validia K.R., Gokani R., 2010. Hepatoprotective and antioxidant activity of root bark of *Calotropis Procera* R.Br (Asclepediacea). International Journal of Pharmacology. 6(6), 937-943.

14. Singh K. K., San E.N., Maheshwari J.K., 2007. Traditional Phytotherapy among the tribe of Varanasi district, Uttarpradesh. Journal of Economic and Taxonomic Botany. 4(3), 8 29-838

15. Harbone J.B., 1976. Methods of plant analysis. In: Phytochemical methods. Chapman & Hall: London. 1976.

16. Olaniyan J.M., Muhammad H.L., Makun H.A., Busari M.B., Abdullah A.S., 2015. Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. Journal of Acute Diseases. 5(1), 62-70.

17. Canadian Council on Animal Care (CCAC), 1997. CCAC guidelines on: animal use protocol review. Ottawa ON: CCAC. pp. 12.

18. Lorke D., 1983. A new approach to practical acute toxicity testing. Archive of Toxicology. 54, 275-287.

19. Adedapo A.A., Sofidiya O.M., Maphosa V., Moyo B., 2008. Antiinflammatory and analgesic activities of the aqueous extract of *Cussonia paniculata* stem bark. Records of Natural Products. 2(1), 43-47.

20. Omulokoli E., Khan B., Chhbra S.C., 1997. Antiplasmodial activity of four Kenyan medicinal plants. Journal of Ethnopharmacology. 56, 133-137.

21. Olorunnisola O.S., Bradley G., Afolayan A.J., 2012. Acute and sub-chronic toxicity studies of methanolic extract of *Tulbaghia violacea* rhizomes in Wistar rats. African Journal of Biotechnology. 11(83), 14934-14940.

22. Organisation for Economic Cooperation and Development. (OECD), 2001. Guidance Document on acute oral toxicity testing. Organization for Economic Co-operation and Development. Paris, France.

23. Amresh G.R., Singh P.N., Rao. V.C., 2008. Toxicological screening of traditional medicine Laghupatha (Cissaampelos pareira) in experimental animals. Journal of Ethnopharmacology. 116, 454-460.

24. Phillip L.W., Robert C.J., Stephen M.R., 2002. Hepatotoxicity: In principles of toxicology, environmental and industrial applications. John Wiley & Sons: New York. pp. 117.

25. Wallace A.H., 2007. Principles and Methods of Toxicology. 5th ed. Healthcare USA, Inc: New York, NY 10017. pp. 369-453.

26. Uma M., Shalimol A., Arumugasamy K., Udhayasankar M.R., Punitha D., 2014. Effect of Methanolic Extract of Smilax Wightii A. Dc. on Serum Protein Profile in Streptozotocin Induced Diabetic Rats. International Journal of PharmTech Research. 6(5), 1746-1750.

27. Adeneye A.A., Ajagbonna O.P., Adeleke T.I., Bello S.O., 2006. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. Journal of Ethnopharmacology. 105, 374-379.

28. Hammami L., Smaoui S., Hsouna A.B., Hamdi N., Trikil M.A., 2015. *Ruta montana* L. leaf essential oil and extracts: characterization of bioactive compounds and suppression of gall disease. Excli Journal. 14, 83-94

29. Asanga E., Edet E.E.P., Eseyin A.O., 2014. Hematological parameters of alloxan-induced diabetic rats treated with ethanol extracts and fractions of *Nauclea lafiloia* leaf. European Scientific Journal. 9(27), 203-210.

30. Garba Y., Abubakar A.S., 2012. Haematological response and blood chemistry of Yankasa rams fed graded levels of *Tamarindus indica* (Tamarind) leaves. Nigerian Journal of Basic and Applied Sciences. 20(1), 44-48

31. Edet E.E., Akpanabiatu M.I., Uboh F.E., Edet T.E., Eno A.E., Itam E.H., Umoh I.B., 2011. *Gongronema latifolium* crude leaf extract reverses alterations in hematological indices and weight loss in diabetic rats. Journal of Pharmacology Toxicology. 6, 174-181.

32. Mayne P.D., 2005. Clinical chemistry in diagnosis and treatment. 6th ed. Amold: London. pp. 280-312.

33. Muhammad S., Hassan G., Dangoggo S.M., Hassan S.W., Umar K.J., Aliyu R. U., 2011. Acute and subchronic toxicity studies of kernel extract of sclerocarya birrea in rats. Scientific World Journal. 6(3), 11-14.

34. Rana S.V., Rekha S., Seema V., 1996. Protective effects of few antioxidants on liver function in rats treated with cadmium and mercury. Indian Journal of Experimental Biology. 34, 177–179.