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Acute and subacute toxicity study of the ethanol crude extract of *Leptadenia hastata* plant (Pers.) Decne. in Wistar rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: From a long time in history medicinal plants have been used traditionally for the treatment of many diseases. The plant *Leptadenia hastata* (Pers.) Decne of the family Asclepiadaceae has been used in the management of many ailments.

Objective: This experimental research was designed to evaluate the toxicity profile of the ethanol whole plant extract of *Leptadenia hastata* in Wistar rats.

Material and Methods: Five groups of rats were used for the toxicity study in which group 1 was used as control while Groups 2, 3, 4 and 5 were administered 200, 400, 800 and 1600 mg/kg of the extract. Subacute toxicity study was carried out for 30 days by oral administration of the different doses of the extract and blood samples were taken for biochemical and haematological investigation. Histopathological study was also conducted on pancreas, liver, kidney and heart.

Results: The LD_{50} for acute toxicity study of the extract was found to be greater than 5000 mg/kg indicating relative safety. From the subacute study, there were no significant changes in the levels of the biochemical and haematological parameters when compared with the control group except for AST, ALP, HCO_3^+, MCV, platelets and platelet width distribution. Likewise, the morphological and histological investigations showed that the colour and architecture of the harvested organs were preserved with no signs of inflammation or distortions in their appearances when compared with the control.

Conclusion: The results of this study indicated that the extract of *Leptadenia hastata* was found to be relatively safe.

Keywords: *Leptadenia hastata*, Acute and Sub-acute toxicity

INTRODUCTION

Medicinal plants have been introduced for the management of many diseases globally due to search for alternative medicine; many patients prefer the use of traditional herbal medicines alone or in combination with conventional agents because of the adverse effects, cost and non-availability of the conventional drugs to majority of the affected populations especially rural areas in developing countries (Botha and Penrith, 2008). However, knowledge about the active constituents of plant preparations is not well defined and information on their toxicity and adverse effects are deficient (Iwalokun *et al.*, 2011). Therefore, based on their traditional use for long periods of time, they are often assumed to be safe (Edziri *et al.*, 2011). Preparations from medicinal plants today constitute an important health problem, in particular their toxicity to the kidneys and liver because many of these medicinal plants have limited data or scientific evidence on their

efficacy and safety (Tulay, 2012). It is generally agreed and frequently considered that medicinal plants and herbal formulations are naturally safer than synthetic drugs (Mahima *et al.*, 2012). However, assumption of this safety should not always be made since a plant may prove efficacious but could have low therapeutic index (Agaie *et al.*, 2007). Some constituents of medicinal plants have been shown to be potentially toxic, mutagenic, carcinogenic and teratogenic (Akintonwa *et al.*, 2009; Abigal *et al.*, 2014). Therefore, it is significant to evaluate the safety of traditional medicines used for treatment of various diseases. *Leptadenia hastata* (Pers.) Decne, belongs to the family Asclepiadaceae and is used as vegetable and medicine by many African populations due to its nutritive and therapeutic properties (Tamboura *et al.*, 2005). The plant has been used ethno-pharmacologically in the treatment of many ailments which include diabetes, cough, sexual impotency, trypanosomiasis, acute rhinopharyngitis, and hypertension as well as antibacterial and antimicrobial (Aliero and Wara, 2009). It is known to contain many phytochemical constituents such as alkaloid, terpenoids, flavonoids, saponins, tannins, cardiac glycoside, cardenolites, carbohydrate, anthraquinones, steroids and phlobatannins (Aliero and Wara, 2009; Bello *et al.*, 2011). An extensive toxicity studies have not been carried out on *Leptadenia hastata* to give assurance of its safety (Tamboura *et al.*, 2005). It is essential to further investigate the toxicity profile of the domestic plants used empirically in order to guarantee their safety and to make available herbal medicinal products that are accessible, affordable, safe, efficacious and subsequently developed in the future into a pure drug entity.

METHODOLOGY

Plant Collection and Identification

The whole parts of the *L. hastata* plant were freshly collected from its natural habitat within water treatment plant, Maiduguri, Borno State, Nigeria in May 2016 based on the report from its ethnomedical survey. The plant was identified and authenticated by a plant Taxonomist at the Department of Biological Science with a voucher number UM/FPH/001/001/001 and was deposited in Pharmacognosy herbarium, University of Maiduguri.

Preparation and Extraction of the Plant Materials

The fresh aerial parts and the root of *L. hastata* were collected and cut into small pieces, shade dried in a ventilated room away from dust and direct sunlight for 10 days, then powdered with a mechanical grinder and the powdered plant material was stored in airtight container kept at room temperature. A total of 2100g of the powdered plant material of *L. hastata* was weighed and extracted with 2.5 liters of ethanol using Soxhlet apparatus in three successive extractions each weighing 700g. Initially, the powdered plant material was defatted using petroleum ether for 48 hours. After defatting, the marc was then allowed to dry and further extracted thoroughly with 95% ethanol using the Soxhlet apparatus. The solvent was removed from the extracts under reduced pressure using rotary vacuum evaporator and further dried in hot air oven at 50°C and kept in an air tight container for further use and analysis**.**

Experimental Animals

A total of 42 apparently healthy Wistar rats (150-170 g) of both sexes were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri for the study. They were checked out for signs of illness and anomalies and were allowed to acclimatize to the laboratory environment for two weeks before they were used for the study. The rats were housed under controlled environmental condition at room temperature and had free access to food and water *ad libitum* according to CIOMS and ICLAS, 2012; the guideline for international guiding principles for biomedical research involving animals and, the procedures and protocols as approved by ethics review committee of the University.

Toxicity Study

Acute toxicity study (oral)

Modified Lorke's method was used to determine the acute toxicity (LD_{50}) of the ethanol crude plant extract of *L. hastata*. Three different doses of the ethanol crude plant extract of *L. hastata* were administered orally to the rats in both phases 1 and 2. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsion, and mortality for 24 hours. Two rats were used in each of the groups in both phases.

Sub-acute toxicity study

A total of 30 rats were randomly divided into five groups of six animals each. Group 1 served as control and received normal saline 2ml/kg while groups 2, 3, 4 and 5 were treated orally with ethanol whole plant extract of *L. hastata* at doses of 200, 400, 800 and 1600mg/kg body weight respectively for thirty days. The test substance was administered daily in graduated doses to different groups of experimental rats, one dose level per group for a period of 30 days.

- Group 1. Distilled water (control).

- Group 2. Ethanol extract of *L. hastata* (200mg/kg).
- Group 3. Ethanol extract of *L. hastata* (400mg/kg).
- Group 4. Ethanol extract of *L. hastata* (800mg/kg).
- Group 5. Ethanol extract of *L. hastata* (1600mg/kg).

Measurement of Body Weight
The body weights of the rats in each group were recorded prior to dosing and then once a week during the dosing periods to observe weight gain or loss.

Haematological and Biochemical Assays
At the end of the 30 days treatment period, blood collected by cardiac puncture into EDTA was analyzed for haematological parameters using the method of Dacie and Lewis (1991) while the blood samples collected in plain bottles were used for biochemical analysis. For biochemical tests, blood collected was allowed to coagulate for 30 minutes at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 minutes and analyzed (Uko *et al.*, 2000; Ihedioha *et al.*, 2014).

Measurement of Organ Weights
Approximately 24 hours after the last administration of ethanol crude plant extract of *L. hastata*, the rats were sacrificed humanely and the blood was allowed to drain out, the liver, kidney, pancreas and heart were identified and the harvested organs were trimmed of any adherent tissue and weighed individually using a metler weighing balance and calculated for relative organ weight ratio given as Relative organ weight ratio = organ weight / body weight x 100.

Histopathological Assays
The liver, kidney, pancreas and heart of rats exposed to different doses (200, 400, 800 and 1600 mg/kg) of the extract and control were identified, trimmed of any adherent tissue, harvested, observed from the sacrificed animals and were fixed in 10% buffered neutral formalin in a separate container for each organ from individual animals. All the tissue samples collected were routinely processed for histopathological analysis; the sections were cut in 5-mm thickness and, stained with hematoxylin and eosin (Singh and Sulochana, 1997) for microscopic examination.

Statistical Analysis of Data
Data obtained from the study were analyzed using one way analysis of variance (ANOVA), simple percentages and bar charts. Tukey's multiple comparisons as *post hoc* test was used to determine the relationship between the variables means. The results obtained were expressed as Mean ± Standard Error of the Mean (SEM) in tables. Statistical Package for Social Sciences (SPSS) version 16 software was used for the analysis. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study of ethanol crude plant extract of *Leptadenia hastata*
The results of the oral acute toxicity (LD_{50}) determination of ethanol whole plant extract of *Leptadenia hastata* showed no death within 24 hours of extract administration in both phase 1 (10, 100 and 1000 mg/kg) and phase 2 (1600, 2900 and 5000 mg/kg) respectively (Table 1).

The effects of oral administration of ethanol crude extract of *Leptadenia hastata* on relative organ bod weights of the rats in sub-acute toxicity
The absolute body weights of the rats recorded in the first, second, third and fourth weeks of the 30 days toxicity study are shown in Table 2. There was a progressive decrease in the absolute body weight of the treated groups compared with the control group. The body weight measurements revealed suppressed weight gain, although the loss in the body weight was not statistically significant ($p>0.05$) and was less than 10%. As such, the changes were not considered toxicologically significant. The organ weights of the harvested pancreas, liver, kidney and heart of the rats exposed to different doses of the ethanol extract of *L. hastata* did not show any significant changes when compared with the control group (Table 2). Similarly, the relative organ body weight ratio of the different harvested organs of the rats exposed to different doses of the ethanol extract of *L. hastata* for 30-day sub-acute toxicity shown in Table 3 revealed no significant decrease and/or increase in their weights when compared to control.

Table 1: Acute toxicity study of ethanol crude plant extract of *Leptadenia hastata*

Experimental Phases	*Dose (mg/kg)*	*Observation of death within 24 hours*
Phase I		
	10	0/2
	100	0/2
	1000	0/2
Phase II		
	1600	0/2
	2900	0/2
	5000	0/2
	LD_{50} = > 5000 mg/kg	

There was no death of the rats in any of the phases during the acute toxicity testing of the experiment. This result indicated that the lethal dose (LD_{50}) of L. hastata is greater than 5000 mg/kg body weight of the expermental rats

Table 2: Effects of *Leptadenia hastata* on the organ weights of the rats in the sub-acute toxicity

	Mean ± SEM				
Dose Parameters (g)	**Control**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**1600 mg/kg**
Liver	5.17 ± 0.34	4.30 ± 0.77	5.05 ± 0.20	4.95 ± 0.23	5.15 ± 0.14
Pancreas	0.26 ± 0.03	0.27 ± 0.02	0.30 ± 0. 02	0. 29 ± 0.01	0.30 ± 0.01
Left kidney	0.40 ± 0.03	0.41 ± 0.03	0.45 ± 0.03	0.41 ± 0.02	0.46 ± 0.03
Right kidney	0.41 ± 0.03	0.43 ± 0.03	0.46 ± 0.03	041 ± 0.02	0.48 ± 0.02
Heart	0.51 ± 0.03	0.45 ± 0.02	0.50 ± 0.01	0.47 ± 0.02	0.47 ± 0.03

Values are expressed as mean ± SEM (n = 6); one-way ANOVA. No significant changes were observed in the Organ weight of the treated rats compared with the control (p>0.05)

Table 3: Effects of oral administration of Leptadenia hastata on the relative organ body weights of the rats in sub-acute toxicity

	Mean ± SEM				
Dose Parameters (g)	**Control**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**1600 mg/kg**
Liver	3.41 ± 0.23	3.57 ± 0.12	3.33 ± 0.10	3.42 ± 0.11	3.52 ± 0.13
Pancreas	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0. 01	0. 21 ± 0.01	0.21 ± 0.01
Left kidney	0.26 ± 0.02	0.29 ± 0.02	0.29 ± 0.03	0.28 ± 0.01	0.31 ± 0.02
Right kidney	0.27 ± 0.02	0.31 ± 0.02	0.31 ± 0.03	0.28 ± 0.01	0.33 ± 0.02
Heart	0.34 ± 0.03	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.02

Values were expressed as mean ± SEM, (n = 6); one-way ANOVA. No significant changes were observed in the relative Organ weight of the treated rats compared with the control (p>0.05)

The effects of ethanol extract of *Leptadenia hastata* on haematological markers of the rats in the sub-acute toxicity

There were no significant changes in the total white blood cells (WBC) count, red blood cells (RBC) count, haemoglobin concentration and packed cell volume of the rats exposed to different doses of *L. hastata* extract (200, 400, 800 and 1600mg/kg body weight) compared with the control. Furthermore, no significant changes were noted in the percentage lymphocyte count, granulocytes count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume compared with the control group ($p>0.05$). However, the result showed a significant decrease in platelet count at the doses of 200, 400 and 1600mg/kg body weight and an increase in the platelet diameter width at 1600mg/kg body weight of the ethanol extract compared with the control. Treatment with the ethanol extract of *L. hastata* also indicated significant decrease in mean corpuscular volume at the doses, 400, 800 and 1600mg/kg body weight (Table 4).

Table 4: Effects of ethanol crude plant extract of *Leptadenia hastata* on haematological indices of the rats in the sub-acute toxicity

	Mean ± SEM				
Dose Parameters	Control	200 mg/kg	400 mg/kg	800 mg/kg	1600mg/kg
WBC (10^9/L)	7.65 ± 0.86	6.43 ± 0.91	7.57 ± 0.54	7.68 ± 1.04	9.90 ± 1.32
LYM (%)	63.72 ± 2.26	67.22 ± 2.53	61.08 ± 2.63	61.82 ± 2.48	62.60 ± 2.28
MID (%)	10.10 ± 0.51	9.00 ± 0.42	12.08 ± 1.45	11.07 ± 0.85	11.38 ± 0.83
GRAN (%)	26.18 ± 2.11	23.78 ± 2.20	26.83 ± 1.69	27.12 ± 1.66	26.02 ± 1.55
RBC (10^12/L)	4.06 ± 0.34	4.48 ± 0.13	4.66 ± 0.11	4.61 ± 0.15	4.59 ± 0.09
MCV (fl)	92.08 ± 0.33	92.55 ± 0.98	89.05 ± 0.71*	89.05 ± 0.43*	88.63 ± 0.99*
MCH (pg)	27.92 ± 0.32	28.58 ± 0.28	27.92 ± 0.25	28.32 ± 0.24	27.68 ± 0.47
MCHC (g/L)	266.83 ± 32.63	308.83 ± 1.19	311.17 ± 3.04	315.33 ± 2.89	310.67 ± 1.86
HGB (g/L)	11.50 ± 1.16	12.83 ± 0.17	13.10 ± 0.19	13.48 ± 0.31	12.90 ± 0.26
HCT (%)	38.25 ± 3.11	41.33 ± 0.65	41.87 ± 0.35	41.45 ± 1.18	40.88 ± 0.42
PLT (10^9/L)	354.83 ± 5.53	257.00 ± 30.15*	249.83 ± 30.02*	386.83 ± 29.17	264.83 ± 26.78*
MPV (fl)	9.75 ± 0.08	9.78 ± 0.05	9.90 ± 0.10	9.83 ± 0.13	9.95 ± 0.09
PDW (%)	9.90 ± 0.12	10.28 ± 0.06	10.28 ± 0.09	10.10 ± 0.09	10.35 ± 0.09*

*Values are presented as mean ± SEM (n = 6); assessed by one-way ANOVA. WBC %, LYM %, GRAN %, RBC, MCV, MCH, MCHC, HGB, HCT, PLT, MPV and PDW represent white blood cell count, percentage lymphocyte count, granulocytes count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hemoglobin concentration, packed cell volume, Platelet count, mean Platelet volume and Platelet diameter width respectively. * = $p<0.05$ compared with the control*

The effects of ethanol crude plant extract of *Leptadenia hastata* on rats' biochemical parameters in the sub-acute toxicity

Changes in the renal function indices revealed no significant alteration in the concentrations of sodium, potassium, chloride, urea and creatinine in the rats exposed to doses of 200, 400, 800 and 1600 mg/kg body weight of the ethanol extract compared with the control except for bicarbonate concentration which presented a significant alteration at the dose of 400mg/kg body weight (Table 5).

There was a significant decrease observed in the serum alanine phosphatase level at the dose of 800mg/kg and conversely a significant increase in serum aspartate

aminotransferase (AST) at the same dose of the extract compared with control. However, no significant changes were observed in the serum alanine aminotransferase (ALT) at all doses of the extract compared with control (Table 5). There were no significant variations observed in the serum levels of triglyceride concentration (TG), high density lipoprotein concentration (HDL) and low density lipoprotein concentration (LDL) of the rats at all doses of *L. hastata* extract when compared with the control (Table 5). Though, a significant decrease was noted in the total cholesterol concentration in the rats exposed to the dose of 1600 mg /kg body weight of *L. hastata* extract.

Table 5: Effects of ethanol crude extract of Leptadenia hastata on rats' biochemical parameters in the sub-acute toxicity study

Dose / Parameters	Control	200 mg/kg	Mean ± SEM 400 mg/kg	800 mg/kg	1600 mg/kg
Na^+ (mMol/L)	144.83 ± 0.31	144.83 ± 0.48	145.17 ± 0.48	145.00 ± 0.37	144.17 ± 1.14
K^+ (mMol/L)	5.40 ± 1.07	6.63 ± 0.23	6.78 ± 0.21	6.82 ± 0.25	6.73 ± 0.0.21
Cl^- (mMol/L)	96.33 ± 0.33	96.67 ± 0.56	98.17 ± 0.48	97.33 ± 0.42	96.67 ± 0.71
HCO_3^- (mMol/L)	20.50 ± 0.22	21.00 ± 0.26	21.83 ± 0.31*	21.00 ± 0.37	21.17 ± 0.40
Urea (mMol/L)	5.80 ± 1.10	6.32 ± 0.91	5.13 ± 0.91	5.92 ± 0.72	7.00 ± 1.02
Creatinine (mMol/L)	69.00 ± 2.07	72.17 ± 2.12	70.33 ± 2.16	70.33 ± 1.89	70.00 ± 1.21
ALP (µ/L)	302.33 ± 25.62	301.17 ± 23.50	282.67 ± 15.017	206.50 ± 22.91*	289.67 ± 8.91
AST (µ/L)	137.83 ± 2.02	157.83 ± 7.60	145.17 ± 8.93	167.33 ± 8.22**	147.33 ± 4.67
ALT (µ/L)	82.83 ± 3.00	90.33 ± 9.82	83.17 ± 4.04	88.00 ± 6.53	86.00 ± 4.18
TC (mg/dl)	1.83 ± 0.06	1.65 ± 0.06	1.68 ± 0.03	1.72 ± 0.09	1.55 ± 0.07*
TG (mg/dl)	1.03 ± 0.11	0.90 ± 0.12	1.03 ± 0.06	1.03 ± 0.08	1.05 ± 0.09
HDL (mg/dl)	1.02 ± 0.04	0.98 ± 0.09	0.95 ± 0.06	0.90 ± 0.07	0.85 ± 0.05
LDL (mg/dl)	0.30 ± 0.06	0.22 ± 0.03	0.22 ± 0.03	0.25 ± 0.043	0.25 ± 0.043

*Values were expressed as mean ± Standard Error Mean (SEM), (n = 6); one-way ANOVA. Na^+, K^+, Cl^-, and HCO_3^- represent sodium, Potassium, chloride and bicarbonate concentration respectively, urea and creatinine concentrations. ALP, AST and ALT represent serum alkaline phosphatase, aspartate aminostrasferase, and Alanine aminotransferase respectively. TC, TG, HDL and LDL represent total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein concentrations respectively. Values marked with * and ** were significantly different at p<0.05 compared with control*

Effects of ethanol crude plant extract of L*eptadenia hastata* on histopathology of the organs of the rats in the sub-acute toxicity

The vital organs (pancreas, liver, kidney and heart) of the rats exposed to orally administered doses of the ethanol extract of *L. hastata* produced no significant morphological and histological changes in the organs compared with the control. The pancreatic tissue composed of exocrine acini along with islets of Langerhans and numerous vascular channels within acini were noted (plates a-e). There was hyperplasia of islet cells (plate d) and numerous eosinophils around blood vessels and islets of Langerhans (plate b). Perivascular infiltration by eosinophils and islet cells hyperplasia persisted in plate d but less eosinophils as in plate c (fig.1) and same features were noticed in plate e (fig.1) while no remarkable cellular changes were noticed. The histologic section of the liver showed a preserved hepatic structural design, with hepatocytes arranged in plates with no vascular congestion. There were no areas of necrosis or haemorrhage, no effects on the limiting plates, portal and central vein, sinusoides and no fatty changes or fibrosis were observed in all the plates f-j (fig.2). However, there were numerous eosinophilic infiltrations around the periportal area. The kidney

tissues shown in plates k-o (fig.3) are composed of glomeruli surrounded by bowman's spaces with the glomeruli and tubules cut in varying planes. The glomeruli showed few messengers cells around glomerular capillary channels that have thin basement membrane. The intercession and interstitial vascular channels are unremarkable and a microscopic examination of the kidney showed no tubular necrosis and glomerular congestion in all the treated groups (plates l-o) when compared with the control (plate k). Compared with the control, there were no noticeable histopathological alterations on the heart tissues at all the doses of *L. hastata* extract. Sections also showed cardiac muscles composed of myocytes with few interspersed vascular channels noted in all the plates p-t (fig.4).

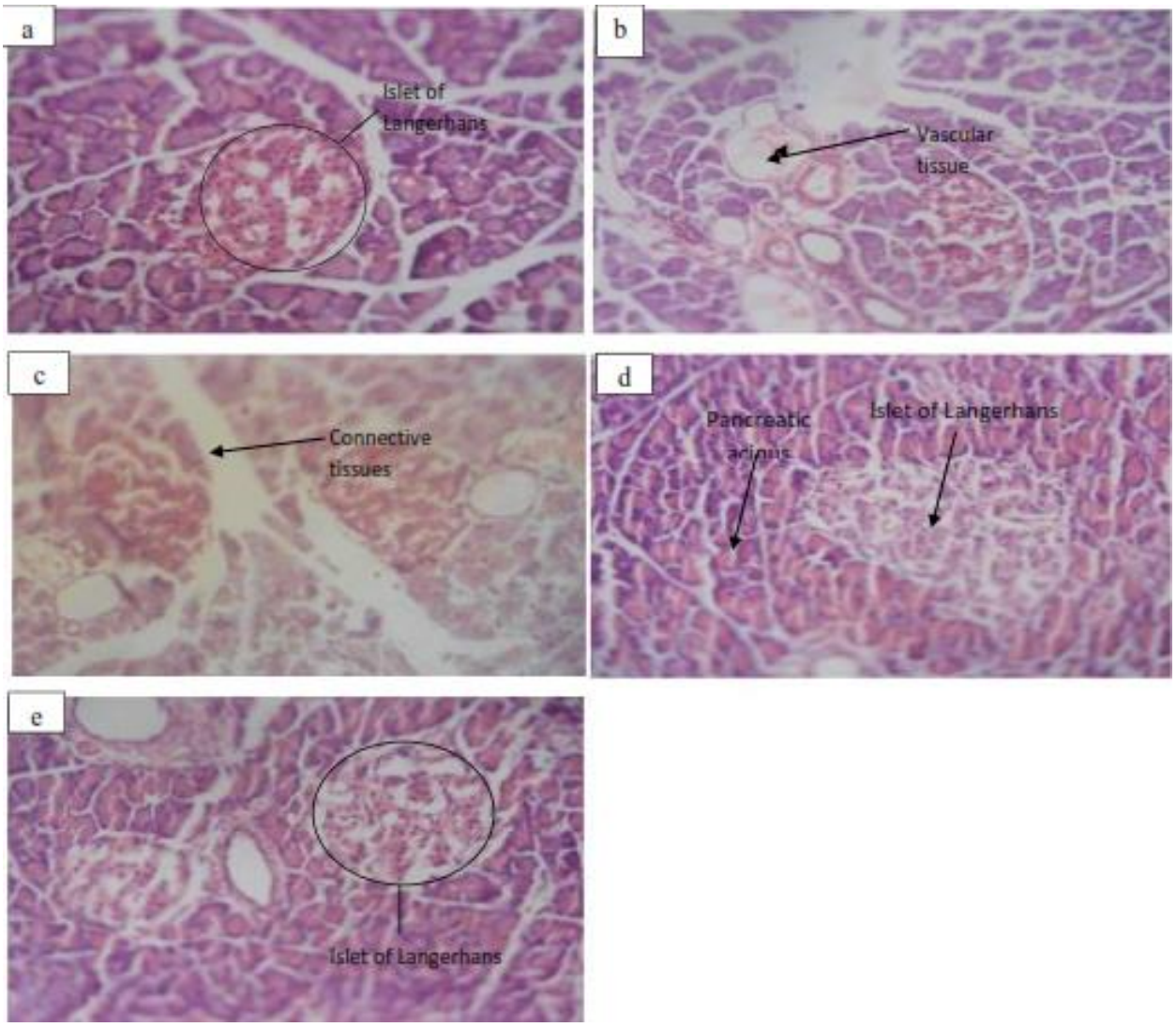

Figure 1: A cross sectional view of pancreas tissue slices from rats treated with ethanolic whole plant extract of L. hastata at doses of (b) 200 mg/kg, (c) 400 mg/kg, (d) 800 mg/kg and (e) 1600 mg/kg bodyweight compared with the control (a) treated with Distilled water, (Magnification, x200)

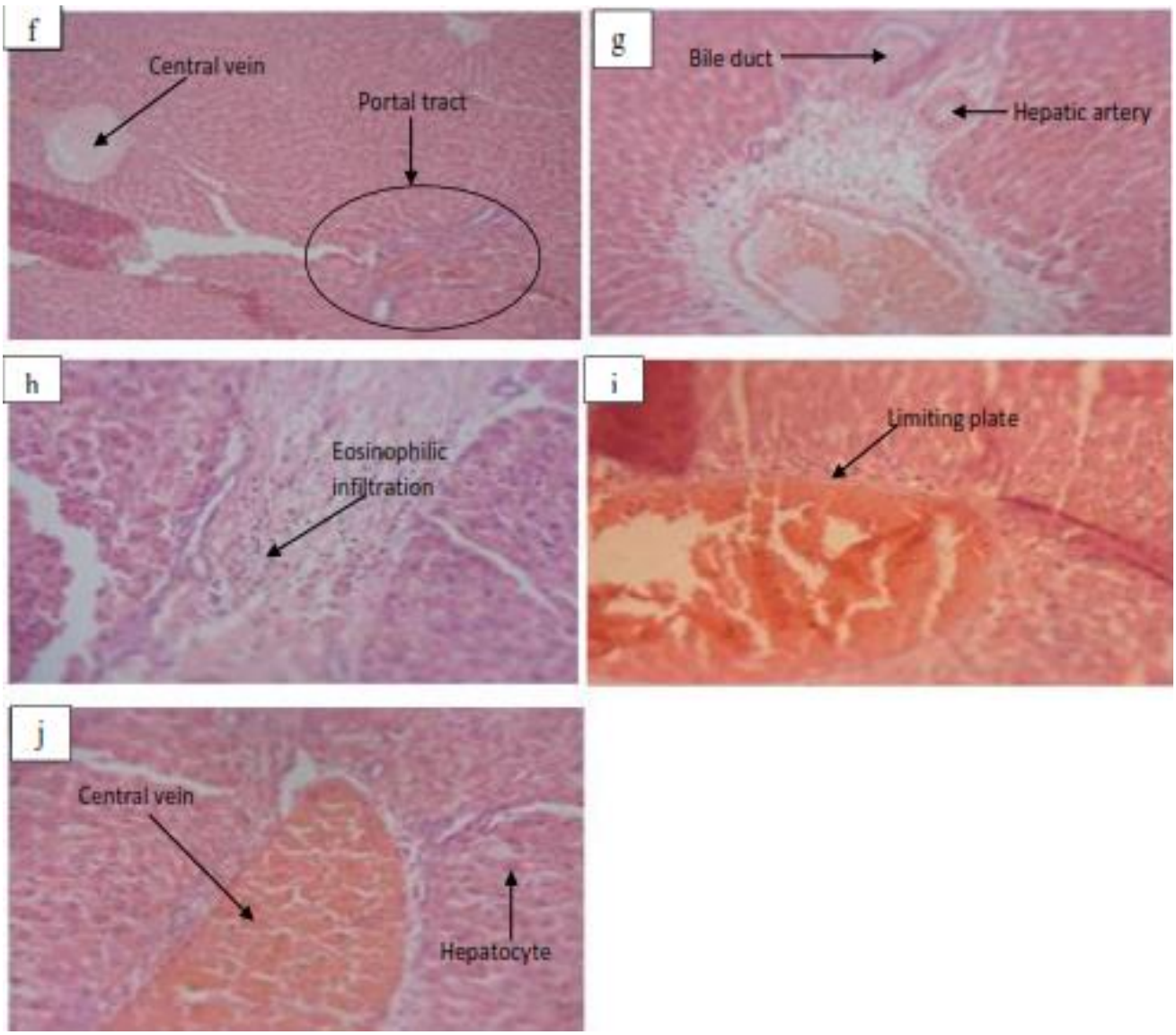

Figure 2: A cross sectional view of liver tissue slices from rats treated with ethanolic whole plant extract of L. hastata at doses of (g) 200 mg/kg, (h)400 mg/kg, (i) 800 mg/kg and (j) 1600 mg/kg bodyweight compared with the control (f) treated with Distilled water (Magnification, x200)

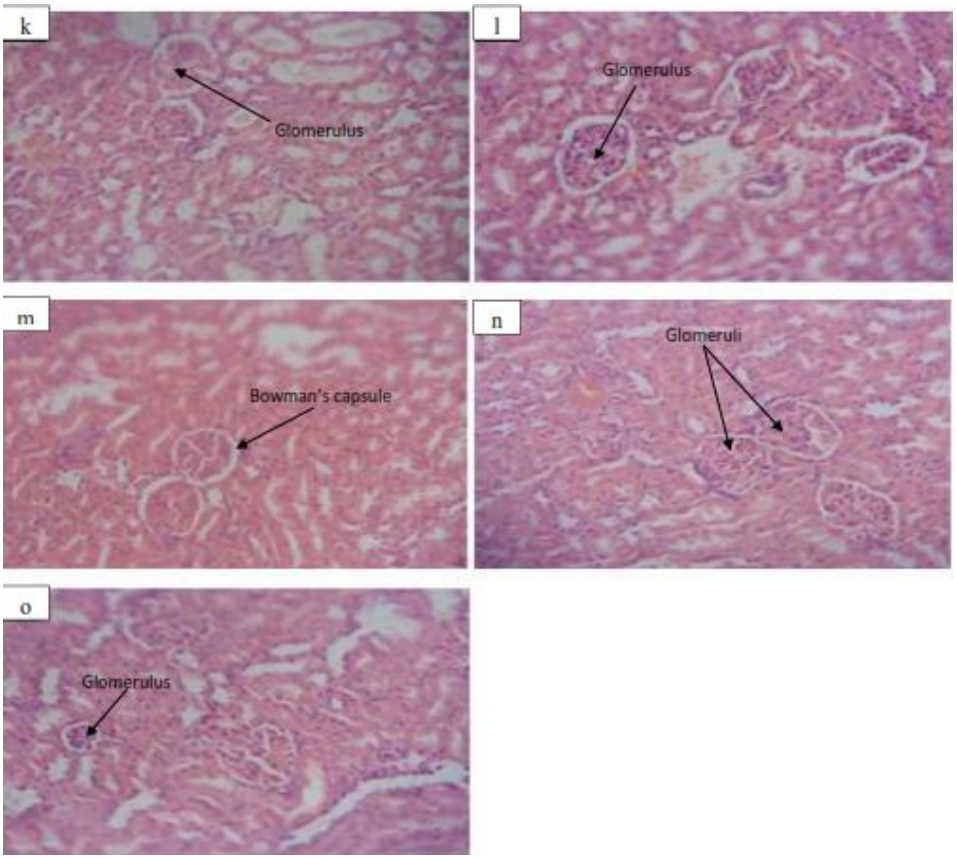

Figure 3: A cross sectional view of kidney tissue slices from rats treated with ethanolic whole plant extract of L. hastata at doses of (l) 200 mg/kg, (m)400 mg/kg, (n) 800 mg/kg and (o) 1600 mg/kg bodyweight compared with the control (k) treated with Distilled water, (Magnification, x200)

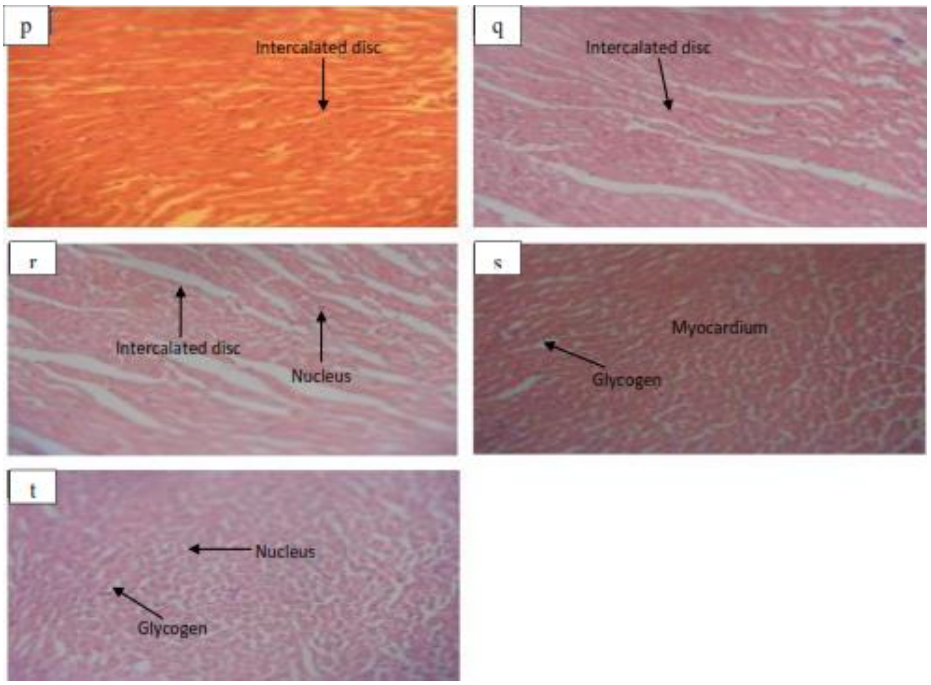

Figure 4: A cross sectional view of heart tissue slices from rats treated with ethanolic whole plant extract of L. hastata at doses of (q) 200 mg/kg, (r)400 mg/kg, (s) 800 mg/kg and (t) 1600 mg/kg bodyweight compared with the control (p) treated with Distilled water, (Magnification, x200)

DISCUSSION

The use of natural indigenous systems of medicine in healthcare systems of any specific country is essential as though the efficacy and safety profile of these medicinal plants need to be evaluated (WHO, 2008). The evaluation of the toxicity profile of a medicinal plant in a subacute study is important for investigating any toxicity on long-term exposure that will give valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at low dose on prolonged exposure that might be concealed and not observed in acute exposure or acutely nontoxic compounds. The oral acute toxicity (LD_{50}) of ethanol crude plant extract of *L. hastata* was found to be greater than 5000 mg/kg, indicating that it is fairly non-toxic.

The *L. hastata* ethanol extract did not show significant effect on experimental animals' gross behaviour except decreased appetite. The rats were alert and responded to physical stimuli during the study although there was a progressive decrease in the body weight of the treated rats throughout the period of the experiment which was not dose dependent. Furthermore, there were no significant changes in the organ weights and also no morphological changes such as inflammation or distortion in the appearance of the organs harvested were observed in the test animals at all doses of *L. hastata* when compared with the control. This suggests non induction of any toxic effect on the kidneys, liver, heart and pancreas since no significant changes were observed in the organ weights of the treated rats compared with the control. This finding is not in conformity with Maina *et al.*, (2013) where there was a steady increase in the mean weight of the liver and kidneys. This could be attributed to the absence of tannins in this study which are known to possess astringent properties that can contribute to increase in organ weight (Maina *et al.*, 2013). The relative organ body weight is fundamental to diagnosing whether the organ was exposed to an injury or not.

Body weight is also an important index of physiological and pathological status in animals (Dybing *et al.*, 2002), because changes in the body weight are indicators of adverse effects of drugs or chemicals (Dybing *et al.*, 2002). The weight loss observed in the body weights of the test animals in this study was not considered significant as the loss was not more than 10% from the initial weight in higher doses (Teo *et al.*, 2002). The loss in body weight of the treated rats might be due to the decreased appetite observed during the study; it could also occur from intestinal damage perhaps from the noxious effect of the plant which consequently decreased food intake (Rabo, 1998); therefore, this suggests that *L. hastata* has altered growth and suppressed weight gain in this study. The loss in the body weight may not be related to organ injury as there was no evidence of injury to the organs of the extract treated rats when compared with the control. Furthermore, no changes in the parameters related to organ toxicity of pancreas, liver, kidney and heart were observed in the hematological and serum biochemical examinations. Therefore, the above-mentioned changes were not considered toxicologically significant (Teo *et al.*, 2002; Hall *et al.*, 2012).

Variations in hematological parameters (red cell indices, total white blood cell count and differentials) could be a pointer to toxicity of plant extracts, for instance reduction of the blood cells suggests a suppression of bone marrow function. The oral administration of the ethanol crude plant extract of *L. hastata* in the subacute toxicity study showed insignificant changes in the white blood cells counts, lymphocyte, granulocytes, red blood cell count, haemoglobin, haematocrit, MCH, and MCHC concentrations in the rats at all doses when compared with the control. This may suggest that it has no toxic effects on leucopoiesis and haemopoiesis as decrease in blood hemoglobin concentration suggests anemia, while a decrease in WBC count suggests immunosuppression and consequent decline in immune system's response to infection or antigens. However, a significant decrease was observed in the platelets (PLT) count at the doses of 200, 400 and 1600 mg/kg body weight indicating that *L. hastata* has an antiplatelet effect, although the reason for this effect is not clear. Furthermore, a decrease in MCV levels at the doses of 400 mg, 800 mg and 1600 mg/kg body weight and an increase in the platelet diameter (PDW) at 1600 mg/kg body weight were observed which were statistically significant when compared with the control.

Biochemical measurements can be used in the diagnosis of toxicity of drugs or medicinal plants on pancreas, liver, heart, and kidney, acid-base imbalance in the respiratory and metabolic systems, lipid metabolism as well as other metabolic disorders. In safety and efficacy evaluation of medicinal plants, serum parameters are important for the identification of hepatocellular injury. An increase in liver enzymes, (AST, ALT and ALP), is most often associated with hepatocellular damage and therefore the results of these enzymes measurement and liver histology has been shown to be crucial for the assessment of hepatic injury in preclinical studies as an indicator of potential liability for hepatic injury in humans (Dufour *et al.*, 2000).

From this study, the serum AST levels was significantly increased at the dose of 800 mg/kg body weight but significantly decreased serum ALP at the

dose of 800 mg/kg body weight and no significant alteration in the levels of ALT at all doses of ethanol extract of *L. hastata* were observed when compared with the control. AST is found in the heart, brain, kidney, skeletal muscle and liver and thus any damage in these tissues can cause increase in serum AST activity (Boyd, 1983). Therefore, the observed increase in the levels of AST at the dose of 800 mg/kg body weight without a concurrent significant increase in ALT may suggests a procedure-related increase (physical and psychological stress), and not due to pathological changes, hepatocellular injury or necrosis of hepatocytes since there was no significant change in ALT levels compared with the control, based on the report of Arakawa *et al* (1997) and Sanchez *et al* (2002). This may also be a response to emotional stress, pre analytical effects (handling) as much as physical perturbations or muscle injury that is not detected by histopathology (Nancy, 2015) which might cause transient increases in ALT and AST. Muscle injury can cause increase in serum transaminase activity by increasing the permeability of the cell membranes, resulting in the release of aminotransferases into the blood stream (Ali *et al.*, 2008) but AST is generally higher than ALT when both are concurrently increased (Meyer and Harvey, 2004).

Several factors might be the cause of discordant finding of increased transaminase values without histologic correlates. The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury, in part because of the longer half-life of ALT and the greater proportion of AST that is bound to mitochondria (Travlos *et al.*, 1996). Furthermore, ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST in rats, dogs, and Non-Human Primates (Boyd, 1983). Hepatic causes of increased serum ALT activity, with or without increased AST activity, include hepatocellular necrosis, injury, or regenerative/reparative activity and can also be affected by extrahepatic factors (Hall, 2001). According to Amacher *et al* (2001) increases in ALT activity also have been reported with concurrent hepatic microsomal induction in the dog and rat but were not considered indicative of hepatic injury because there were no substantive concurrent changes in liver histology or liver weight as observed in this study. Increase in ALP levels indicates nonspecific tissue irritation because of the potential contribution of extrahepatic factors and cellular damage in numerous organs. Factors such as increase in intestinal or bone isoenzyme activity, hepatobiliary pathology and bone growth/disease are associated with increased serum ALP activity (Meyer and Harvey, 2004; Amacher *et al.*, 2001).

There were no observable changes in the serum triglyceride concentration (TG), high density lipoprotein concentration (HDL) and low density lipoprotein concentration (LDL) at all doses of *L. hastata* compared with the control. The slight variations were found to be insignificant ($p>0.05$). The total cholesterol concentration was significantly decreased in rats treated with the highest dose; 1600 mg /kg body weight of *L. hastata* compared with the control. There were no significant changes in the concentrations of sodium, potassium, chloride, urea and creatinine in the treated groups at the doses of 200, 400, 800 and 1600 mg/kg body weight compared with the control. Therefore, the variation in serum biochemical parameters were statistically not significant in the renal function test in comparison with the control group except a significant increase in bicarbonate concentration at the dose of 400 mg/kg body weight which might not be clinically significant. According to Bailey *et al* (2012), functional studies in toxicological investigations should be coupled with the appropriate histological studies because appropriate morphological studies are useful, especially during the anatomical localization of the action of a toxin. Based on this, a histological study of the effect of ethanol extract of *L. hastata* was conducted. The histopathological investigation of the pancreas showed normal histological features of the pancreatic tissues at all dose levels used, composed of exocrine acini with numerous vascular channels and distinctive islets of Langerhans. There were no remarkable cellular abnormalities observed except that there were perivascular infiltration by eosinophils in the treated/test groups compared with the control which could be as a result of allergic reactions (plate a) because eosinophilic organ infiltration is often associated with a parasitic infection, drug hypersensitivity and collagen vascular disease (Roufosse and Weller, 2010). It can also occur without eosinophilia or direct parasite infection (Jang *et al.*, 2014). The eosinophilic infiltration in the test groups is mildest at the dose of 400 mg/kg (plate c), though there were no significant differences in the eosinophilic infiltration among the treated groups (fig. 1).

The liver tissues of treated rats showed a preserved hepatic structural design with numerous hepatocytes arranged in plates with no vascular congestion, detrimental histologic changes, degeneration, fatty changes or fibrosis, areas of necrosis or haemorrhage. There were no effects on the limiting plates, portal vein, central vein and sinusides observed (plates g-j) when compared with control group (plate f) (fig.2); however, there were numerous eosinophilic infiltrations around the periportal area. The kidney tissues of treated rats showed normal glomeruli

surrounded by bowman's spaces, and tubules cut in varying planes. The glomeruli revealed few messengers cells around glomerular capillary channels that have thin basement membrane. The interstial vascular channels are remarkable in all the plates and there is no tubular necrosis and glomerular congestion (fig.3). The histopathological studies showed normal cardiac muscles at all doses tested (fig.4). All these showed that the extract has no significant structural changes in the major organs of the body.

CONCLUSION

The evaluation of the ethanol crude plant extract of *L. hastata* revealed no sign of toxicity or mortality. The oral acute toxicity (LD_{50}) value of the ethanol crude plant extract *of L. hastata* was found to be greater than 5000 mg/kg body weight. The 30-day subacute toxicity study of the extract at all dose levels tested revealed the harmless nature of this plant on hepatic, renal and haemopoetic system even at the highest dose used. The gross examination and the histopathological analysis of liver, pancreas, heart and kidney showed no toxic effects or any abnormalities induced by *L. hastata* extract in the test rats. This is further anchored by no significant changes of the ethanol extract of *L. hastata* on biochemical and haematological indices in the study. Therefore, based on the present assessments and investigations, the study has shown that the ethanol crude plant extract of *L. hastata* can be said to be relatively safe buttressed by the findings of Tamboura *et al.* (2005) which stated that *L. hastata* is safe.

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