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TOXICITY ASSESSMENT OF ETHANOL EXTRACT OF *SAUSSUREA LAPPA* (COSTUS) ROOT IN MALE WISTAR RATS

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ABSTRACT: ***Background and Objective:*** *Saussurea lappa is a medicinal plant that has been used in the treatment of various disease conditions for decades. Researchers have provided diversified characteristics of this medicinal plant, enormous data about the bioactive compounds and as well its pharmacological properties. However, information regarding toxicity study of Saussurea lappa root is lacking. The acute and sub-chronic toxicity of Saussurea lappa root ethanolic extract was investigated in this study using an in vivo model (male Wistar rats).* ***Materials and Method:*** *The acute toxicity study was done in two phases: Phase One comprised four groups of three rats each which were administered with 50 mg, 100 mg and 1600 mg per kg bodyweight ethanolic extract of S. lappa while the last group (control) was given water and normal fed once. Phase Two involved administering 1600 mg, 2900 mg and 5000 mg per bodyweight. The median lethal dose of the plant was determined in the acute toxicity study. In sub-chronic toxicity studies, the effects of S. lappa root ethanol extract in daily single oral administration at the doses of 200, 400 and 800 mg/kg during 28 days were determined. The blood haematological and biochemical parameters as well as the histopathological examination of the liver and kidneys were studied.* ***Results:*** *In acute study, a single administration of the Saussurea lappa root ethanolic extract up to a dose of 5000 mg/kg did not cause mortality of animals all through the study. Thus, the median lethal dose (LD_{50}) of Saussurea lappa root ethanolic extract was greater than 5000 mg/kg. Saussurea lappa root ethanolic extract caused no significant effect in the haematological and biochemical parameters of the treated groups when compared to the control. There were also no noticeable histological changes in the liver and kidneys of the Saussurea lappa treated rats compared to controls*.. ***Conclusion:*** *These results have shown that oral administration of Saussurea lappa root ethanol extract did not yield any significant toxic effect when acutely and daily administered for 28 days in the male Wistar rats. Hence, Saussurea lappa root ethanol extract could be regarded as a safe natural product for therapeutic use.*

KEYWORDS: *Saussurea lappa*, Wistar rat, toxicity, ethanolic extract, lethal dose, histology, haematological.

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INTRODUCTION

Medicinal plants have been an important source of cure for a long period in almost all cultures globally. The majority of the orthodox medicines are made from medicinal plants, which are regarded as rich sources of traditional remedies [1]. More than 80% of individuals worldwide use medicinal plants in the management of their ailments. This has led to much concern about the toxicity and safety effects of these medicinal plants, mostly indicated by traditional healers. Plants contain hundreds of constituents and some of them may elicit toxic side effects. Several studies exist reporting the toxic effects of herbal medicines [2].

Saussure*a lappa*, or costus root, is a medicinal plant that grows in the Himalayan region and belongs to the family Asteraceae [3]. It comprises various active components with medicinal properties that are attributed to the presence of flavonoids, steroids, terpenes, alkaloids, sesquiterpenes, costunolide, dehydrocostus lactone, cynaropicrin, and chlorogenic [4,5].

Saussurea *lappa (S. lappa)* has the potential to treat various disease conditions in allopathic and herbal medicine such as asthma, chronic skin disease, inflammatory diseases, ulcer, typhoid fever, cough and cold, toothache, leprosy, and stomachache, among others [6]. It is one of the antioxidant-rich medicinal plants with various bioactive properties such as anticancer, anti-ulcer, anti-inflammatory, hepatoprotective, immunomodulator, hypoglycemic, spasmolytic, anticonvulsant, antidiarrheal and antiviral activities [7]. However, there is a lack of evidence-based data on the toxicity of *Saussurea lappa* root. Therefore, the current study was undertaken to determine the possible toxic effect of ethanol extract of *Saussurea lappa* root in male Wistar rats.

MATERIALS AND METHOD

Experimental Animals

The experiment was conducted on Wistar rats (*Rattus norvegicus*) weighing between 150 g to 200 g obtained from the animal facility center of Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The animals were acclimatized for a period of two (2) weeks before the commencement of the study. Standard commercial chow and water were provided ad libitum for the animals. Housing conditions were maintained at 25 ± 2 °C at 12 h day/night cycles. The care and handling of the animals were according to the established public health guidelines in Guide for Care and Use of Laboratory Animals (2011).

Plant Collection and Identification

The dried roots of Saussurea *lappa* (costus root) were purchased from New Delhi, India. Taxonomic identification and authentication of the plant was carried out by the taxonomist from the department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen (**PCG/UDUS/ASTE/0003**) of the plant was deposited at the herbarium of the same department.

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Preparation and Extraction of Plant Material

The dried root was mechanically powdered and stored in an air-tight container for further processing. The ethanolic extract of dry powdered roots was prepared according to the modified method of [8], that is, 500 g of the crude powder of Saussurea *lappa* was soaked in 2.5 liters of 70% ethanol with continuous stirring for 72 hours at room temperature, and the mixture was filtered using filter paper (Whatman number 1, England). The ethanolic filtrate was evaporated at 45°C under reduced pressure using a rotary evaporator, the aqueous residues were removed through lyophilization process by freeze drier and the stock extract was stored at -20 °C. The dry extract was subjected to in vitro measurements before its administration into the Wistar rats.

Toxicity Studies

Acute Toxicity

Acute toxicity was performed in accordance with Lorke's method. It was conducted in two phases. Phase One was conducted using nine (9) male Wistar rats, which were divided into three groups of three animals each. Each group was administered ethanolic extract of *Saussurea lappa* at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg per body weight respectively. The animals were placed under observation for 24 hours to monitor their behavior as well as if mortality will occur. In the absence of mortality, Phase Two was proceeded into. This phase was conducted using three Wistar rats, which were distributed into three groups of one rat each. The rats were administered higher doses (1600, 2900 and 5000 mg/kg) of ethanolic extract of *Saussurea lappa* and then observed for 24 hours for behavior as well as mortality [9]. After 24 hours, no mortality was observed and, as such, the LD50 of the *S. lappa* is considered greater than 5000 mg/kg.

Sub-chronic Toxicity Study

Sub-chronic toxicity study was conducted according to OECD 425 guidelines [10]. Twenty-five (25) male Wistar rats were divided into five (5) groups of five (5) rats per group. Group 1 served as the control and received distilled water as a vehicle. Graded doses of crude extract were administered orally to the rats in groups as 2, 3, 4 and 5. The doses given to the groups were 200 mg/kg, 400 mg/kg, 800 mg/kg and 1000 mg/kg body weight daily for 28 days respectively. All the rats had free access to feed and water throughout the period of the experiment and they were observed daily for behavioral changes, changes in the skin and fur, feeding pattern and weight. On Day 29, the rats were weighed and sacrificed. Blood samples were collected via cardiac puncture for biochemical and haematological analyses. The liver samples were excised and fixed in formalin solution for histological analysis.

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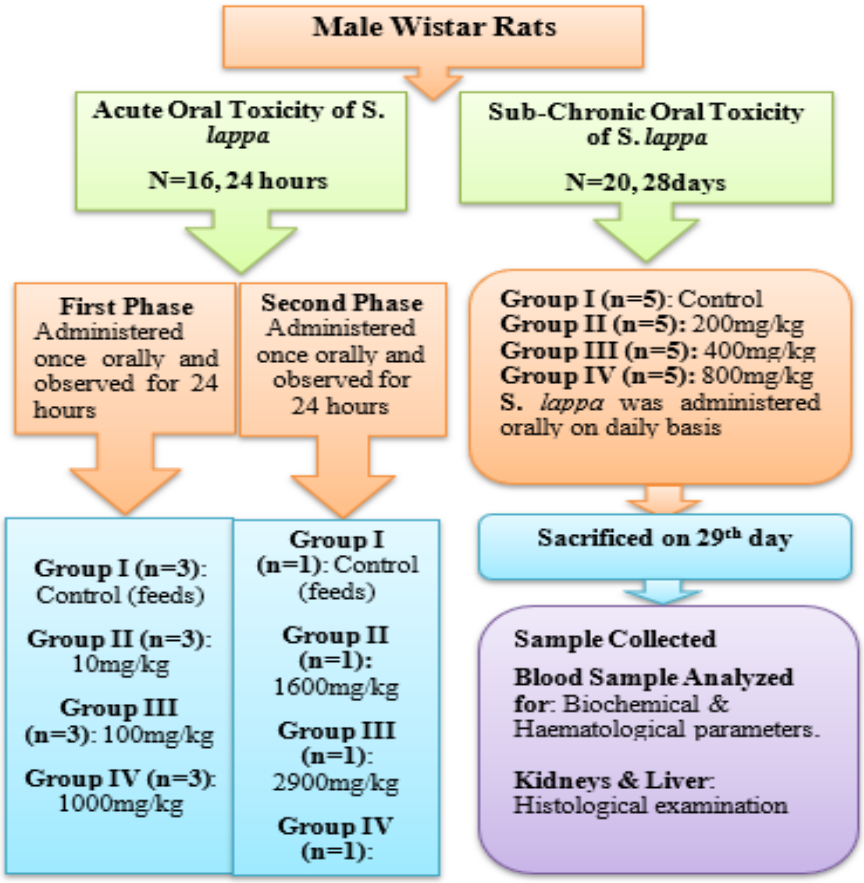

Fig 1: Experimental flow chart of the toxicity study

Haematological Assay

Automated haematology analyzer (Mythic 22 CT, country USA) was used to determine the complete count such as red blood cells (RBC), white blood cells (WBC), haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), etc from an EDTA collected blood.

Biochemical Analysis

For biochemical analysis, blood without additives was centrifuged at 4000 rpm at 4°C for 10 min to collect the serum and tests were done using biochemical analyzer Robonik (Robonik India Pvt. Ltd., New Mumbai). Biochemical studies were carried out for liver function test (in terms of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and kidney function test (urea and creatinine levels).

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Histopathological Examination

For histopathological studies, the liver and kidney were dissected out from the animals of each group and washed with normal saline and immediately fixed in 10% formalin. The organs were dehydrated in gradual grades of ethanol (50–100%), cleared in xylene and embedded in paraffin. Sections of 3–5 μm thickness were prepared using a rotary microtome, processed in alcohol-xylene series, stained with haematoxylin and eosin (H–E) dye and observed under microscope (Nikon Eclipse 80i, Tokyo, Japan).

Statistical Analysis

Data generated from the study were analyzed using the statistical package for social sciences (SPSS) version 29.0.1.0. Results were expressed as mean ± SEM (standard error of mean). The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test. The differences in values of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Acute Oral Toxicity Study

The results of acute oral toxicity (LD_{50}) are revealed below. Neither death nor symptoms of adverse effects were recorded during the course of the study. There were no significant changes observed in physical parameters such as changes in the fur, mucous membrane of the eye, behavioral patterns, tremors, salivation and diarrhea on the rats in all treated groups as compared to the control group after 24 hours, 72 hours and up to 14 days post oral treatment. This is shown in Table 1.

Table 1: Acute oral toxicity study of ethanolic extract of *Saussurea lappa* roots in male Wistar rats

Groups	Doses (mg/kg)	Observation Period	Behavioral Changes	Mortality
Phase 1		Up to 14 days		
1: 3 rats	10		None	None
2: 3 rats	100		None	None
3: 3 rats	1000		None	None
Control	0			
Phase II				
1: 1 rat	1600		None	None
2: 1 rat	2900		None	None
3: 1 rat	5000		None	None
	LD_{50}= >5000mg/kg			

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Parameter	Control (n=3)	200mg/kg (n=3)	400mg/kg (n=3)	800mg/kg (n=3)
RBC ($x10^{12}$/L)	5.53±0.96	6.74±0.09	6.84±0.14	6.71±0.32
HCT (%)	30.23±5.82	35.93±1.57	35.3±0.68	37.13±1.53
HGB (g/dl)	12.07±2.13	14.67±0.47	14.5±0.15	14.5±0.61
MCV (fl)	54.3±1.47	53.3±2.05	51.67±1.85	53.64±0.78
MCH (pg)	21.8±0.20	21.2±0.51	21.23±0.55	21.63±0.15
MCHC (g/dl)	40.23±0.85	39.73±0.68	41.03±0.38	39.03±0.12
WBC ($x10^{9}$/L)	25.4±12.97	23.07±5.8	20.9±1.99	28.2±2.72
LYM (%)	75.37±6.34	79.16±2.43	74.83±3.18	78.13±3.9
NEU (%)	12.46±1.69	9.03±0.17	15.86±2.47	15.86±2.47
MON (%)	10.4±5.63	9.76±2.64	6.96±0.73	7.8±0.8
EOS (%)	0.43±0.18	0.30±0.06	0.47±0.14	0.27±0.07
BAS (%)	1.33±0.27	1.73±0.29	1.87±0.22	1.53±0.27
PLT ($x10^{9}$/L)	418±50.95	470.67±16.83	491±17.08	386.33±50.01

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 (LD50) of *Saussurea lappa* is greater than 5000 mg/kg body weight of the experimental rats.

The Effect of Ethanol Extract of *Saussurea lappa* on Haematological Parameter in the Sub-chronic Toxicity Study

There were no significant changes in the complete blood count parameters of the rats exposed to different doses of ethanol extract of *Saussurea lappa* roots (200, 400, 800 mg/kg body weight) compared with the control ($p>0.05$) as revealed by one-way ANOVA (Table 2).

Table 2: The effect of ethanol extract of *Saussurea lappa* on haematological parameter in the sub-chronic toxicity study

Values are expressed in mean ± SEM. RBC = Red Blood Cell, HCT = Haematocrit, HGB = Haemoglobin, MCV = Mean Cell Volume, MCH = Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Concentration, WBC = White Blood Cell, LYM = Lymphocytes, NEU = Neutrophil, MON = Monocytes, EOS = Eosinophil, BAS = Basophil, PLT = Platelets, SEM = Standard error of mean, fL = femtoliters, g/dL = gram per deciliter, pg = picogram, /L = Litre. $p\leq0.05$ was considered statistically significant.

The Effect of Ethanol Extract of *Saussurea lappa* on Biochemical Parameter in the Sub-chronic Toxicity Study

The renal function indices revealed no significant alteration in the concentrations of urea and creatinine in the rats exposed to doses of 200, 400 and 800 mg/kg body weight of the ethanol

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extract compared with the control. However, the liver enzymes revealed no significant difference in the serum alanine phosphatase, aspartate aminotransferase (AST) and alanine

Dose **Parameters**	**Normal Control**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Urea (mg/dL)	5.8±0.60	6.1±0.75	5.37±0.46	5.53±0.57
Creatinine (mg/dL)	0.52±0.04	0.57±0.40	0.54±0.02	0.60±0.07
AST (IU/L)	138.33±19.64	123±7.37	124±0.57	133.33±9.87
ALP (IU/L)	237.67±20.21	240.33±16.1	259.33±16.74	261±13.00
ALT (IU/L)	155±8.66	159±6.76	154.33±7.79	156±3.13

aminotransferase (ALT) activity at doses of 200, 400 and 800 mg/kg body weight of the ethanol extract compared with the control (Table 3).

Table 4.3: The effect of ethanol extract of *Saussurea lappa* on biochemical parameter in the sub-chronic toxicity study (n=3)

Values are expressed in mean ± SEM. AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, mg/dL = microgram per deciliter, IU/L = International unit per liter.

Histopathological Examination

Histopathological examinations of liver section in the Wistar rats treated with ethanol extract of *Saussurea lappa* root are shown in Figure 2. Microscopic observation of liver sections of normal control revealed healthy hepatic cells without fatty lobulation, well-preserved cytoplasm, prominent nucleus and intact central vein (Figure 2A). Rats administered with ethanol extract of *Saussurea lappa* root 200, 400 and 800 mg/kg showed normal liver architecture with no necrosis (Figure 2B–2D).

However, the histopathological examination of kidney sections from Wistar rats treated with ethanol extract of *Saussurea lappa* root is shown in Figure 3. The kidneys from rats administered with distilled water (control group) demonstrated intact glomeruli and tubules (Figure 3A). Rats administered with ethanol extract of *Saussurea lappa* root 200, 400 and 800 mg/kg showed a normal architecture structure similar to the control group (Figure 3B–3D).

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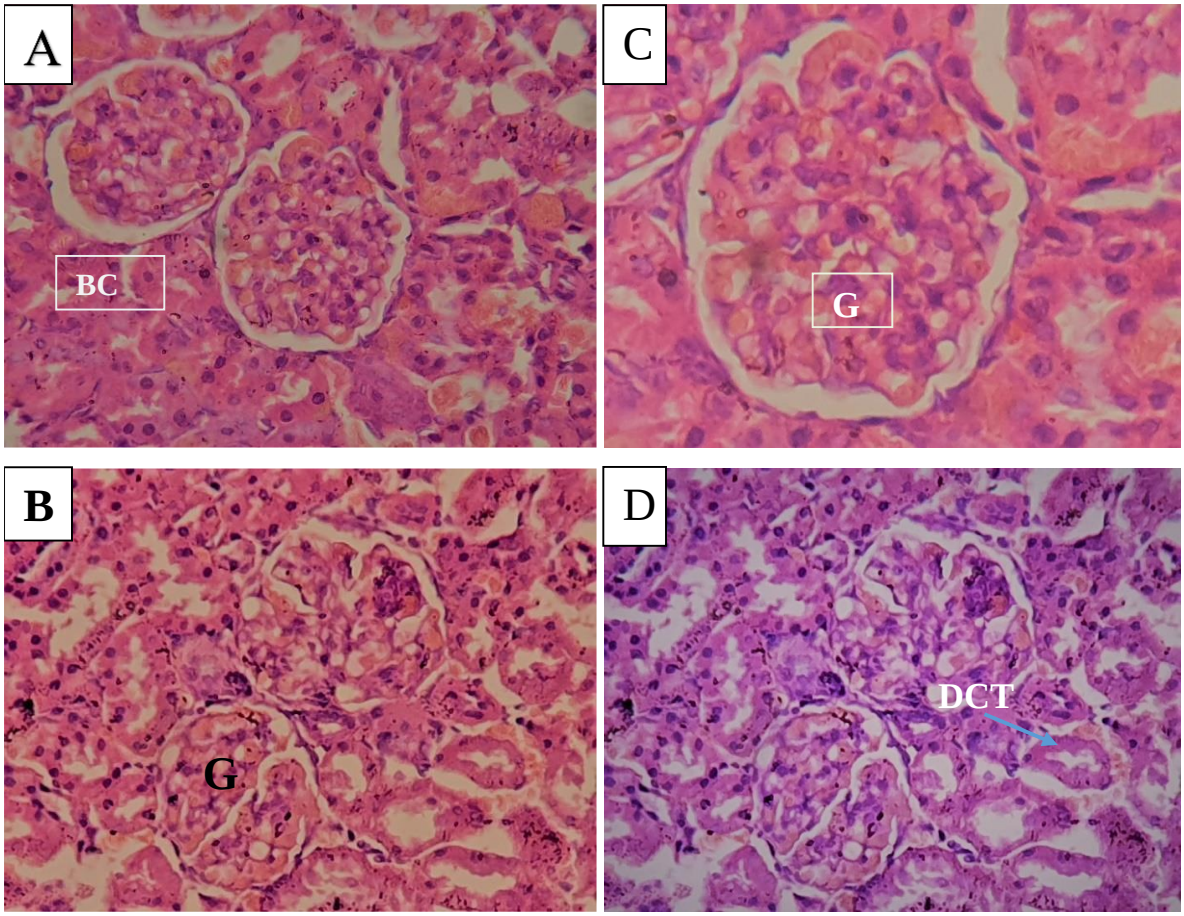

Figure 2: A cross sectional view of kidney tissue slices from Wistar rats treated with ethanolic extract of Saussurea lappa at doses of (B) 200 mg/kg, (C)400 mg/kg and (D) 800 mg/kg bodyweight compared with the control (A) treated with Distilled water, (H & E Stain, Magnification, x400). G = glomerulus, DCT= distal convulated tubule and BC= bowman capsule.

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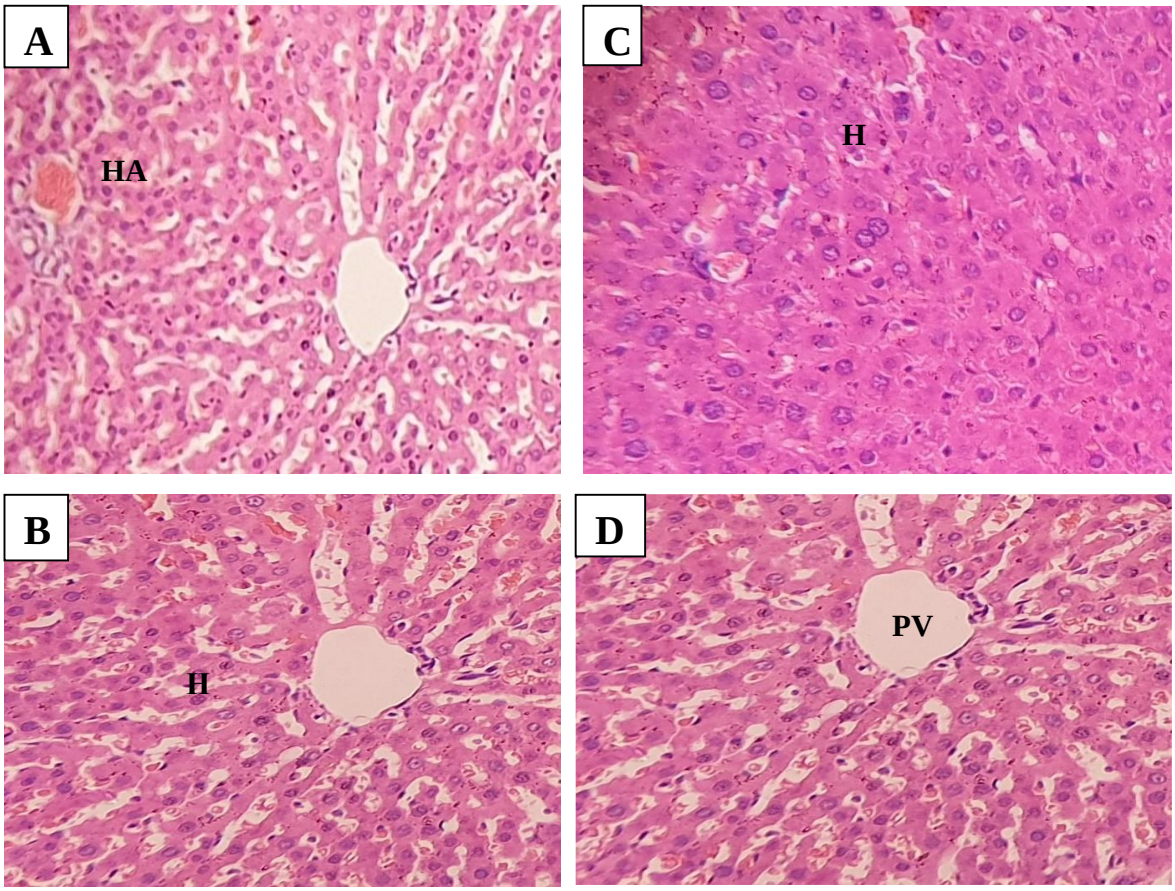

Fig 3: A cross-sectional view of liver tissue slices from Wistar rats treated with ethanolic extract of *Saussurea lappa* root at doses of (b) 200 mg/kg, (c) 400 mg/kg and (d) 800 mg/kg body weight compared with the control (a) treated with distilled water (H & E Stain, Magnification, x400). H = hepatocytes, PV = portal vein and HA = hepatic artery.

DISCUSSION

In this present study, ethanol extract of *Saussurea lappa* root showed no toxic effect in the Wistar rats all through the study. In the acute toxicity study, the plant extract caused no signs of toxicity such as tremor, nausea, diarrhea, convulsion, death and changes in the fur, skin and eye color. The Median Lethal Dose (LD_{50}) of *Saussurea lappa* root ethanolic extract was greater than or equal to 5000 mg kg, which means that the plant extract at the highest dose of (5000 mg kg) administered is not toxic after single oral administration to Wistar rats. However, this finding is in agreement with the Hodge and Sterner toxicity scale level 5 which classified medicinal plants as practically non-toxic with LD_{50} higher than 5000 mg/kg body weight when administered.

In the sub-chronic toxicity studies, *Saussurea lappa* root ethanolic extract caused no significant ($p<0.05$) effect on the haematological and biochemical parameters in the treated groups when compared to the control group. The haematological parameters such as RBC, MCV, MCH,

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MCHC, HCT, HGB, WBC, LYM, MON, NEU, EOS, BAS and PLT were significantly compared to the control group.

In the present study, no significant effect was observed in the serum activity of the liver enzymes, such as AST, ALT, ALP and serum markers of the kidney, urea and creatinine. This finding revealed that the consumption of *S. lappa* for long duration is not associated with derangement in the haematological indices and some biochemical markers.

Based on the histological examination, all tissues of the kidneys and liver presented good structures; no cellular lesions and inflammation were found upon microscopic examination. There were no significant changes when comparing both the slides of the organs from treated and control groups. This suggests that the ethanolic extract of Saussurea *lappa* does not affect the kidneys and liver of the Wistar albino rats. From this study, it was suggested that the ethanolic extract of *Saussurea lappa* does not have adverse effects on the kidneys and liver of the Wistar albino rats. The present findings may justify the use of Saussurea *lappa* by traditional healers in Northern Nigeria in the treatment of various diseases.

CONCLUSION

The evaluation of ethanol extract of *Saussurea lappa* root can be considered safe as it did not exhibit any lethality nor adverse effects in male Wistar rats. The oral acute toxicity (LD50) value of the ethanol extract *of Saussurea lappa* root was found to be greater than 5000 mg/kg body weight. The 28 day sub-chronic toxicity study of the extract at all dose levels tested revealed the harmless nature of this plant on hepatic, renal and haemopoietic systems even at the highest dose used. The gross examination and the histopathological analysis of the liver and kidney showed normal appearance in the treated groups when compared to the control group. The present study provides supportive data on the use of *Saussurea lappa* in traditional medicine and could lend credence for its medicinal use. Chronic toxicity, mutagenicity and carcinogenicity evaluations should be performed to have a better understanding of the plant's safety profile.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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