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Toxicological evaluation of the ethanol extract of *Lophira alata* (Ochnaceae) stem bark in rats

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Abstract
Though *Lophira alata* is used in ethnomedicine for the management of a host of central nervous and systemic diseases, the toxicity profile has not been investigated. This study evaluated the toxicological profile of the ethanol extract of *Lophira alata* stem bark in rats. The mean lethal dose was determined. Animals were treated with 50-800 mg/kg of the ethanol extract for four weeks after which body weight/organ ratio, haematological, biochemical (renal and hepatic) and lipid profile of test animals were evaluated. Changes in body weight and histopathological examination of brains, kidneys and livers were also carried out. Lower doses (50-400 mg/kg) did not affect body weight, organ/body weight ratio, hepatic, renal, haematological, lipid profile and morphology of organs of test animals. The highest dose affected creatinine levels, serum proteins and induced some histopathological changes. *Lophira alata* is relatively safe at low doses but high doses of *Lophira alata* should be used with caution.

Keywords: *Lophira alata;* Toxicity; Lipd profile; Haematological indices

INTRODUCTION

Leaves, stem barks and roots of plants in form of decoctions, infusions or solvent extracts are used for the management of a wide range of disease conditions in many countries of the world as alternatives to orthodox Medicine. Factors responsible for this include availability of these plants, folkloric use, cost and associated low incidence of adverse effects [1,2]. Data on long and short-term toxicity resulting from the use of plants in ethnomedicine is often not available or documented. Such data is often useful for establishment of valuable information such as safe doses, potential organ damage or teratogenic effects [3,4].

Lophira alata popularly known as 'iron red wood' or 'ekki' in South Western Nigeria is a woody tree found in many countries in Africa. Extracts of different parts of *Lophira alata* are used ethnomedicinally in parts of Africa for the management of toothache, hepatic infections, female sterility, pyrexia, sepsis and for respiratory infections [5,6]. An aqueous infusion of the stem bark of *Lophira alata* and *Afzelia africana* is administered orally as a sedative and anti-psychotic agent [7]. While the seeds, root, leaves and stem bark are used as anti-aging agent in the South Western part of Nigeria, [8,9] the stem bark is used in the management of epilepsy [10].

Falade *et al*. [11] evaluated the anti-malarial activity of hexane, methanol and ethyl acetate leaf extract of *Lophira alata* extract *in vivo* and *in vitro* models of malaria.

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Studies by Iniaghe *et al.* [12] revealed the antidepressant, anxiolytic and anti-epileptic properties of the aqueous extract of *L. alata* stem bark.

Studies on the toxicity profile of *Lophira alata* to the best of our knowledge have not been carried out, we therefore aim to evaluate the toxicity profile of the ethanol extract *Lophira alata* stem bark in rats.

EXPERIMENTAL

Plant materials. Stem bark and leaves of *Lophira alata* obtained from Okeigbo Village in Ondo State in April, 2018 were identified and authenticated by Mr. Adeniji of the Forestry Research Institute of Nigeria, Ibadan where a voucher specimen was deposited for future reference. The stem barks were thoroughly cleaned, air dried and ground in a mill to obtain the powdered form. Three litres of Ethanol was added to 1 kg of the powdered extract and the mixture was left for 72 hours at room temperature with gentle stirring. Thereafter separation was carried out using filter paper (12-15 µm) and concentrated on the rotary evaporator set at 40°C. The resultant concentrate was dried overnight in an oven. Product obtained (dark brownish non-sticky, crystalline-like compounds) was transferred to smaller vessels and stored at 4°C in the refrigerator.

Phytochemical screening. Phytochemical screening of the extract was carried out using the methods described by Trease and Evans [13]. The extract was screened for the presence of alkaloids, tannins, saponins, anthraquinone glycosides, steroids, flavonoids and cardiac glycosides.

Animals. Adult albino mice (18-25 grams) and Wistar rats (120 -200 grams) procured from the Department of Pharmacology and Toxicology, University of Benin, Nigeria were used for the study. The animals were kept in polypropylene cages at the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Nigeria. They were maintained under standard laboratory conditions and were fed with standard laboratory animal feed and clean water *ad libitum*. Handling of the animals was done according to standard protocols for the use of laboratory animals of the National institute of Health [14]. Institutional approval was obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria.

Acute toxicity study. The acute toxicity of *L. alata* stem bark extract was determined in mice using the method described by Lorke with some modifications [15]. Briefly, nine mice randomly distributed into three groups of three animals per group, were treated with 10, 100 and 1000 mg/kg of *L. alata respectively* via the oral route and observed for signs of toxicity and death for 24 hours. In the second phase of the acute toxicity study, nine animals in groups of 3 animals per group received 1600, 2900 and 5000 mg/kg the extract and observed for number of deaths for 24 hours. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which no animal died.

Sub-acute toxicity study. Adult Wistar rats were randomly divided into six groups of eight rats each. They were treated daily for 28 days with 50-800 mg/kg of *Lophira alata* stem bark extract orally; control group received distilled water [16]. Body weights were recorded on the first day of the study and every four days for the entire period of the study. After the 28-day treatment, animals were sacrificed under chloroform anesthesia, blood was collected via the abdominal aorta and transferred to EDTA and plain tubes respectively for haematological and biochemical analysis. The brains, lungs, hearts, right kidneys, livers and spleen of test animals were also carefully isolated weighed and used to calculate the relative organ weight ratio of each animal. Thereafter these organs

were preserved in 10% formo-saline solution and used for histopathological analyses.

Organ to body weight ratio. The organ to body weight ratio was calculated using the formula
(Weight of Organ / Weight of animal on the day of sacrifice) × 100 [17].

Haematological analysis. Blood collected in EDTA tubes was used for the following assays white blood cells, lymphocytes, monocytes, granulocytes, red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpusular haemoglobin mean corpuscular haemoglobin concentration, red blood cell distribution width, plateletcrit, platelet count, mean platelet volume and platelet distribution width [17].

Biochemical analysis. Blood samples transferred to plain tubes were allowed to clot and then centrifuged at 4000 rpm for ten minutes. The sera were carefully separated and transferred to fresh plain tubes and used for biochemical analysis. Biochemical indices assayed are liver function tests viz alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, total bilirubin, conjugated bilirubin; kidney function tests namely urea, creatinine, and serum electrolytes- sodium, potassium, bicarbonate and chloride ions [17] and lipid profile - total cholesterol, total triglycerides, high density lipoprotein, low density lipoproteins.

Statistical analysis. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test using Sigma Stat® version 11. A difference was considered significant at $p<0.05$. The results are presented as mean ± standard error of mean (SEM).

RESULTS

The percentage yield from extraction was 12.83 %.

Phytochemical screening. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroids, anthraquinone glycosides, steroids, carbohydrates, flavonoids and cardiac glycosides. Results of phytochemical screening are presented in Table 1.

Effects of ethanol extract of *L. alata* stem bark on body weights and organ/body weight ratio. There was an increase in body weights for both control and treatment groups compared to baseline values from the eighth day of the study. There was however no significant difference in body weights of treatment groups when compared to animals in the treatment group (Table 3). There was also no significant difference in organ/weight ratio between control and *L. alata* treated animals (Table 4).

Effects of ethanol extract of *L. alata* stem bark on haematological parameters. Treatment with *Lophira alata* at dose levels of 200-800mg/kg caused a slight decrease in monocyte count; however these were not significantly different from the control group. There was no significant differences in other haematology parameters between the control and *L. alata* treatment groups. Data is presented in Table 5.

Effect of *L. alata* on liver function test indices. While the highest dose level caused a significant decrease in total and conjugated bilirubin levels, no significant difference in other hepatic indices was observed in other hepatic indices of both L. alata treated animals and animals in the control group (Table 6).

Effect of *L. alata* on kidney function test indices. A significant decrease in creatinine levels was observed in animals treated with the highest dose of *Lophira alata.* No

significant difference was observed in other hepatic parametres. Data is presented in Table 7.

Effect of *L. alata* on lipid profile of test animals. No significant different was observed in cholesterol, triglycerides, high and low density proteins in both *L. alata* treated and control animals (Table 8).

Histology and morphology of brains, livers and kidneys of rats treated with different doses of *L. alata.* No significant histological changes were noticeable in the brains of rats treated with 50-400 mg/kg *L. alata.* Treatment with the highest dose (800 mg/kg) showed evidence of thickened dilated white matter. Representative photographs are shown in figures 1A-F.

In hepatocytes, pathological changes were not seen in sinusiods of animals treated with lower doses (50-200 mg/kg) of the extract. Scanty mononuclear cells, hepatocytes with slightly vacuolated nucleus and mild steatosis were observed in 100, 200 and 400 mg/kg dose levels while mild fibrosis and stenosis was observed in the 800 mg/kg treatment group (figures 2A-F).

Renal corpuscle, interstitial spaces and tubules of control and 50 mg/kg treatment group were morphologically intact while mild diffused inflammatory cells and mild tubular necrosis was observed in the 100 and 400 mg/kg dose level. In the 800 mg/kg treated animals, atrophied renal corpuscles, interstitial space and tubules were noticeable. Representative micrographs are shown in figures 3A-F.

DISCUSSION

In the present study, we evaluated:
(i) phytochemical components of the ethanol extract of *Lophira alata* stem bark
(ii) acute and sub-acute toxicity of *Lophira alata* in mice
(iii) changes in body weight and organ weight/ratio (iv) Haematological, hepatic and renal indices (v) Lipid profile (vi) histo-pathological changes in brains, hearts, spleens, liver, lungs and kidneys of animals treated for four weeks with ethanol extract of *Lophira alata* stem bar*k.*

Table 1: Phytochemicals present in ethanol extract of *L. alata* stem bark

Phytoconstituents	
Alkaloids	+
Tannins	+
Saponins	+
Anthraquinone glycosides	+
Steroids	+
Carbohydrate	+
Flavoniods	+
Cardiac glycosides	+

+, present

Table 2: Oral lethal dose (LD_{50}) of ethanol extract *L. alata* stem bark in mice

Groups/Phases	n	Dose (mg/k)	Mortality (%)
Phase 1			
1	3	10	0
2	3	100	0
3	3	1000	0
Phase 2			
1	3	1600	0
2	3	2900	0
3	3	5000	0

Table 3: Effects of *L. alata* on body weight of animals post sub-acute administration

	D1	D4	D8	D12	D16	D20	D24	D28
Control	116.15 ± 1.67	121.92 ± 1.84	135.93 ± 1.85*	139.67 ± 2.41*	145.42 ± 2.16*	151.55 ±3.35*	152.98 ± 3.48*	162.43 ±3.92*
LA 50 mg/kg	133.13 ± 5.16	135.55 ± 4.76	148.28 ± 5.89	154.25 ± 5.61*	163.35 ± 6.51*	173.20 ± 5.38*	176.23 ± 5.80*	186.45 ± 6.35*
LA 100 mg/kg	145.49 ± 8.90	151.31 ± 8.85	161.63 ± 8.19	171.86 ± 7.24*	172.52 ± 6.91*	181.47 ± 7.60*	182.56 ± 6.92*	191.43 ± 7.23*
LA 200 mg/kg	138.70 ± 1.58	141.88 ± 1.60	153.67 ± 2.07	153.70 ± 2.08*	163.09 ± 1.83*	165.60 ± 1.73*	168.24 ± 1.79*	177.85 ± 2.15*
LA 400 mg/kg	131.33 ± 2.58	134.97 ± 2.83	144.88 ± 2.55	145.22 ± 2.38	146.33 ± 1.73	151.20 ± 2.44	154.18 ± 2.60*	161.98 ± 3.08*
LA 800 mg/kg	115.23 ± 2.41	120.73 ± 2.27	126.00 ± 1.98	128.00 ± 1.77	135.35 ± 1.08*	138.20 ± 8.13*	141.95 ± 4.42*	144.61 ± 2.04*

Body weights of animals treated with ethanol extract of *Lophira alata*. LA represents *Lophira alata*, D for days of treatment. Data is expressed as mean ± SEM. *p<0.05 compared to baseline values; n=5-8 per group

Table 4: Effects of *L. alata* on organ/weight ratio of animals post sub-acute treatment

Treatment	Brain	Heart	Lungs	Liver	Right kidney	Spleen
Control	0.92 ± 0.03	0.40 ± 0.01	080 ± 0.03	4.16 ± 0.11	0.33 ± 0.01	0.46 ± 0.03
LA 50 mg/kg	0.87 ± 0.05	0.38 ± 1.02	0.77 ± 0.05	3.94 ± 0.06	0.35 ± 0.01	0.49 ± .005
LA 100 mg/kg	0.76 ± 0.03	0.35 ± 0.01	0.86 ± 0.05	3.89 ± 0.09	0.31 ± 0.01	0.46 ± 0.01
LA 200 mg/kg	1.04 ± 0.09	0.38 ± 0.01	0.93 ± 0.03	3.57 ± 0.05	0.33 ± 0.01	0.48 ± 0.01
LA 400 mg/kg	0.86 ± 0.05	0.37 ± 0.01	0.86 ± 0.04	4.23 ± 0.11	0.34 ± 0.01	0.59 ± 0.05
LA 800 mg/kg	0.98 ± 0.11	0.38 ± 0.04	0.72 ± 0.01	4.28 ± 0.01	0.31 ± 0.01	0.55 ± 0.08

Organ/weight ratio of animals treated with ethanol extract of *Lophira alata*. LA represents *Lophira alata*. Data is expressed as mean ± SEM; n=5-8 per group

Table 5: Effect of *L. alata stem bark* on Haematological Indices

Haematological Indices	Control	LA 50 mg/kg	LA 100 mg/kg	LA 200 mg/kg	LA 400 mg/kg	LA 800 mg/kg
WBC	11.32 ± 0.58	8.33 ± 0.27	10.40 ± 0.44	9.95 ± 0.41	9.03 ± 0.28	10.55 ± 2.02
LY (*10^8)µl	6.08 ± 2.02	6.01 ± 0.70	7.37 ± 0.26	5.85 ± 0.15	5.32 ± 0.19	6.55 ± 1.45
MO (*10^8)µl	3.37 ± 0.54	4.23 ± 1.40	3.54 ± 0.41	1.85 ± 0.13	1.56 ± 0.08	1.80 ± 0.21
GR (*10^8)µl	4.12 ± 0.45	3.73 ± 0.83	3.30 ± 0.21	2.33 ± 0.13	2.10 ± 0.21	2.20 ± 0.35
LY%	45.03 ± 3.41	38.27 ± 9.30	48.01 ± 3.77	59.92 ± 0.76	59.55 ± 2.61	61.35 ± 2.09
MO%	15.95 ± 1.25	13.43 ± 3.68	12.41 ± 1.18	18.05 ± 0.45	17.50 ± 0.77	17.60 ± 1.56
GR%	27.50 ± 1.81	22.27 ± 2.19	20.17 ± 0.73	22.03 ± 0.38	22.95 ± 1.91	21.05 ± 0.53
RBC (*10^6)µl	7.05 ± 0.18	7.15 ± 0.12	6.64 ± 0.11	6.95 ± 0.13	6.33 ± 0.37	6.77 ± 0.21
Hg g/dl	8.82 ± 0.36	9.90 ± 1.04	8.70 ± 0.41	8.18 ± 0.15	7.68 ± 0.34	7.05 ± 0.04
HCT%	39.37 ± 1.23	39.97 ± 1.14	36.43 ± 0.43	39.62 ± 0.68	36.65 ± 1.09	36.80 ± 0.50
MCV fl	55.53 ± 0.77	54.43 ± 0.98	55.56 ± 0.95	57.17 ± 0.66	59.38 ± 2.17	54.45 ± 2.37
MCH pg	12.63 ± 0.60	13.83 ± 1.52	13.13 ± 0.73	11.73 ± 0.07	11.63 ± 0.28	10.40 ± 0.28
MCHC g/dl	22.73 ± 0.95	24.77 ± 2.56	23.49 ± 1.08	20.63 ± 0.20	20.83 ± 0.44	19.10 ± 0.35
RDW%	21.97 ± 0.69	21.50 ± 1.87	29.07 ± 2.06	20.43 ± 0.24	20.75 ± 1.05	19.65 ± 0.60
PLT%	669.67 ± 16.87	550.00 ± 8.82	506.33 ± 39.42	546.00 ± 44.18	661.25 ± 51.11	578.00 ± 71.12
PCT%	0.40 ± 0.03	0.42 ± 0.02	0.36 ± 0.02	0.37 ± 0.01	0.28 ± 0.02	0.37 ± 0.01
MPV fl	3.75 ± 0.25	4.33 ± 0.68	3.93 ± 0.33	3.08 ± 0.02	3.25 ± 0.09	3.00 ± 0.01
PDW fl	6.73 ± 0.31	7.87 ± 0.05	6.64 ± 0.43	7.22 ± 0.10	8.00 ± 0.38	6.80 ± 0.28

Haematological parametres of animals treated with ethanol extract of *Lophira alata*. Data is expressed as mean ± SEM; n=5-8 per group. LA represents *Lophira alata*, White Blood Cells (WBC), lymphocytes (LY), monocytes (MO), granulocytes (GR), red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpusular haemoglobin (MCH), mean corpuscular haemoglobin concentration

(MCHC), red blood cell distribution width (RDW), plateletcrit (PLT), platelet count (PCT), mean platelet volume (MPV) and platelet distribution width (PDW). * $p<0.05$ compared to the control group

Table 6: Effect of *L. alata stem bark* on liver function

Hepatic Indices	Control	LA 50 mg/kg	LA 100 mg/kg	LA 200 mg/kg	LA 400 mg/kg	LA 800 mg/kg
ALP (µ/l)	201.50 ± 8.98	252.33 ± 22.17	190.71 ± 8.28	192.00 ± 6.65	204.50 ± 12.03	193.00 ± 28.91
ALT (µ/l)	61.67 ± 2.65	55.00 ± 3.53	60.14 ± 1.59	54.00 ± 2.02	61.25 ± 2.21	58.50 ± 0.35
AST (µ/l)	146.5 ± 5.65	151.67 ± 14.49	153.71 ± 5.50	178.86 ± 9.13	146.00 ± 4.85	160.00 ± 1.41
TP (g/dl)	6.10 ± 0.10	5.17 ± 0.12	6.31 ± 0.07	5.97 ± 0.07	6.43 ± 0.07	6.75 ± 0.10
ALB (g/dl)	3.02 ± 0.09	3.03 ± 0.18	3.40 ± 0.05	3.01 ± 0.05	3.48 ± 0.08	3.80 ± 0.07
GLO (g/dl)	3.08 ± 0.03	2.93 ± 0.16	2.91 ± 0.05	2.96 ± 0.03	2.95 ± 0.07	2.95 ± 0.18
TB (mg/dl)	0.37 ± 0.01	0.33 ± 0.04	0.31 ± 0.02	0.37 ± 0.01	0.35 ± 0.01	0.20 ± 0.14*
CB (mg/dl)	0.17 ± 0.01	0.20 ± 0.03	0.13 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.10 ± 0.01*

Hepatic indices of animals treated with ethanol extract of *Lophira alata*. Data is expressed as mean ± SEM; n=5-8 per group. LA means *Lophira alata*, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLO), total bilirubin (TB), conjugated bilirubin (CB). * $p<0.05$ compared to the control group

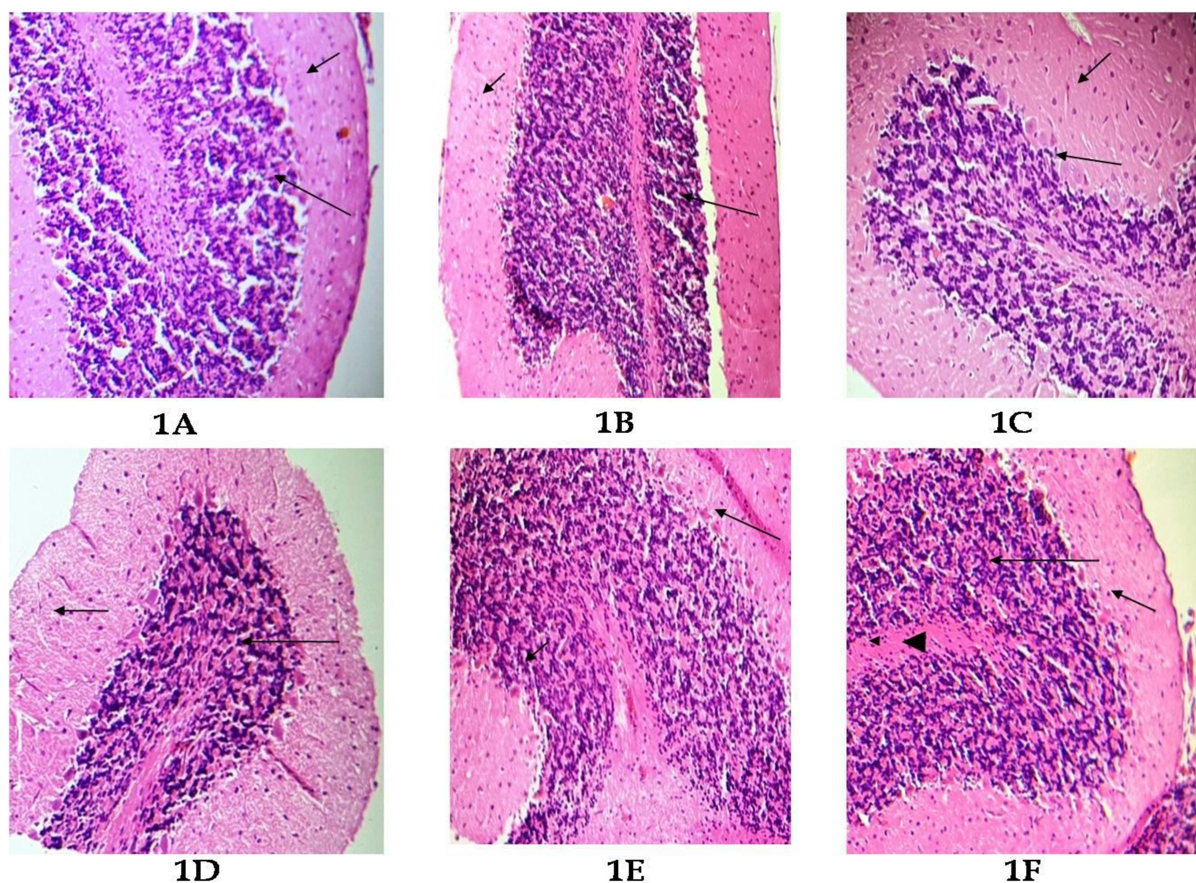

Figure 1: Representative Micrographs of Brains of Rats Treated with *L. alata*

Brain reveals visible detailed features, molecular layer (short arrow) and granule layer (long arrow) bound with prominent Purkinje cell layer (1A);molecular layer (short arrow) and granule layer (long arrow) split through with a narrow white matter bound with prominent Purkinje cell layer (1B); molecular layer (short arrow) and granule layer (long arrow) which appears basophilic, visible Purkinje cell layer (1C); molecular layer (short arrow) and granule layer (long arrow) bound with prominent pyknotic cells (1D); slightly thickened molecular layer (short arrow) and granule layer (long arrow) 1E; molecular layer (short arrow) and granule layer (long arrow) bound with prominent pyknotic cells with thickened dilated white matter (arrow head) 1F. 1A represents the control group while figure 1B-F represent 50, 100, 200, 400 and 800 mg/kg dose levels of *Lophira alata* respectively.

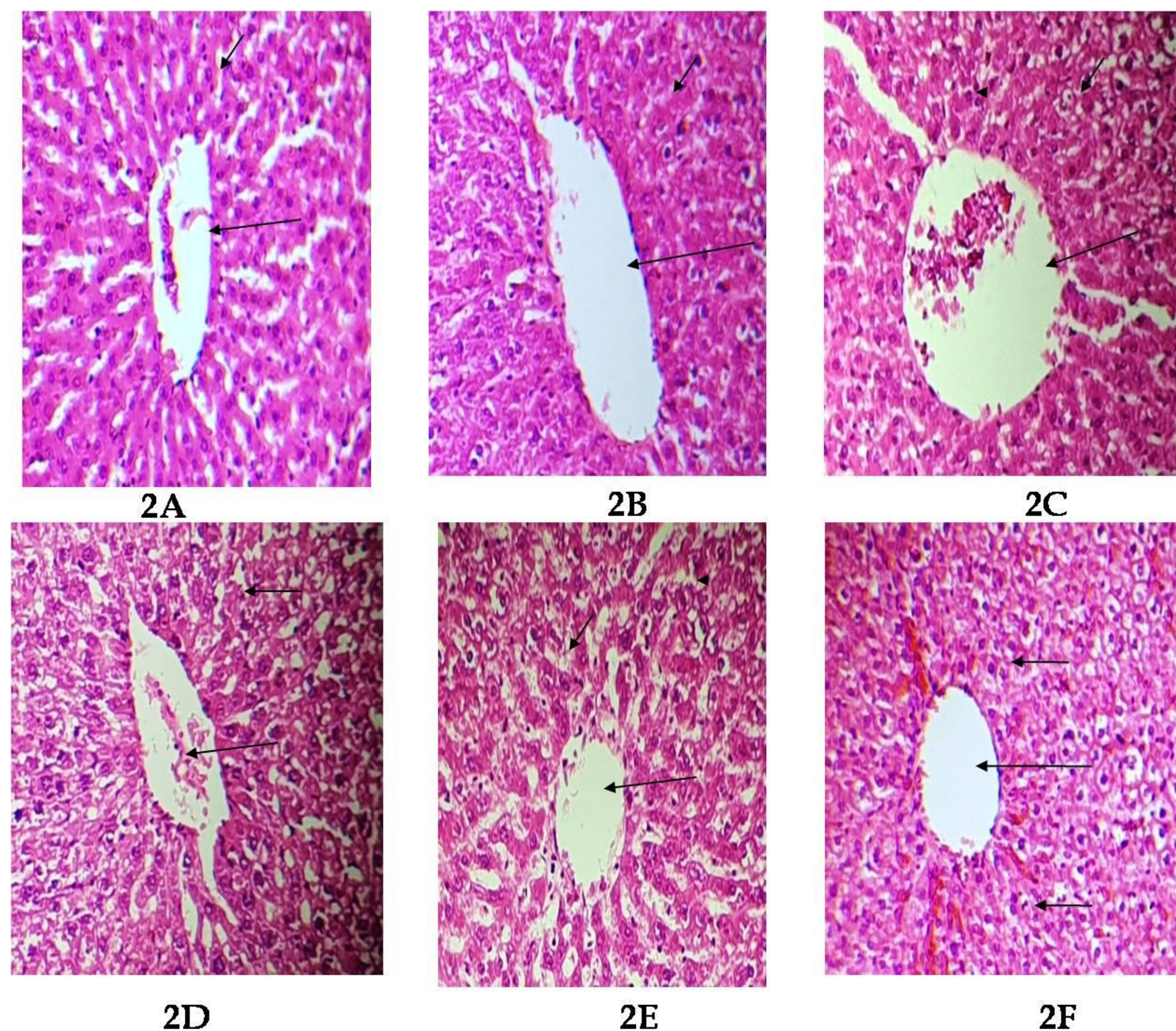

Figure 2: Representative Micrographs of Livers of Rats Treated with *L. alata*
Distinct centriole (long arrow) and hepatocytes with pyknotic nucleus (short arrow) and well fenestrated sinusoids of control group (2A); 50 mg/kg treatment group (2B); scanty mononuclear cells (arrow head) 100 mg/kg treatment group (2C); hepatocytes with slightly vacuolated nucleus (short arrow) and well fenestrated sinusoids 200 mg/kg treatment group (2D); scanty mononuclear cells and mild steatosis (arrow head) 400 mg/kg treatment group (2E); mild fibrosis hepatocytes with pyknotic nucleus with mild fatty changes (short arrows) and visible stenosis 800 mg/kg treatment group (2F).

Table 7: Effect of *L. alata stem bark* on Kidney Function

Renal Indices	Control	LA 50 mg/kg	LA 100 mg/kg	LA 200 mg/kg	LA 400 mg/kg	LA 800 mg/kg
Urea (mg/dl)	42.33 ± 1.34	40.00 ± 1.53	45.43 ± 1.22	43.27 ± 0.80	44.00 ± 2.37	37.50 ± 4.60
Creatinine (mg/dl)	0.77 ± 0.02	0.63 ± 0.04	0.75 ± 0.01	0.71 ± 0.02	0.73 ± 0.04	0.55 ± 0.11*
Sodium (µmol/dl)	139.17 ± 0.85	138.67 ± 1.35	141.43 ± 0.57	142.71 ± 0.46	140.75 ± 0.23	136.50 ± 1.06
Potassium(µmol/dl)	5.75 ± 0.14	5.30 ± 0.20	6.33 ± 0.22	6.14 ± 0.08	6.05 ± 0.09	6.20 ± 1.13
Bicarbonate (µmol/dl)	22.00 ± 0.51	19.67 ± 0.51	22.14 ± 0.32	22.00 ± 0.30	21.5 ± 0.25	18.50 ± 0.35
Chloride (µmol/dl)	102.67 ± 0.87	105.00 ± 0.33	104.43 ± 0.41	106.43 ± 0.41	107.00 ± 0.87	107.51 ± 1.06

Renal indices of animals treated with ethanol extract of *Lophira alata*. Data is expressed as mean ± SEM; n=5-8 per group. LA stands for *Lophira alata*; urea (ur), creatinine (Cr). * $p<0.05$ compared to the control group

Table 8: Effect of *L. alata stem bark* on Lipid Profile

Lipids (mg/dl)	Control	LA 50 mg/kg	LA 100 mg/kg	LA 200 mg/kg	LA 400 mg/kg	LA 800 mg/kg
TCHOL (mg/dl)	80.17 ± 1.27	72.67 ± 1.58	79.57 ± 1.17	81.86 ± 0.72	88.50 ± 1.64	84.00 ± 9.90
TG (mg/dl)	109.67 ± 5.02	97.67 ± 5.97	109.06 ± 4.16	99.00 3.87	96.50 ± 5.26	122.50 ± 13.79
HDL (mg/dl)	42.5 ± 0.71	35.00 ± 1.86	42.00 ± 1.45	44.43 ± 0.58	50.75 ± 1.09	41.00 ± 9.49
LDL (mg/dl)	16.33 ± 0.54	18.00 ± 1.20	14.86 ± 0.56	17.57 ± 0.77	18.50 ± 1.36	18.50 ± 3.89

Lipid profile of animals treated with ethanol extract of *Lophira alata*. Data is expressed as mean ± SEM; n=5-8 per group. LA represents *Lophira alata*; total cholesterol (TCHOL), total triglycerides (TG), high density lipoprotein (HDL), low density lipoproteins (LDL).

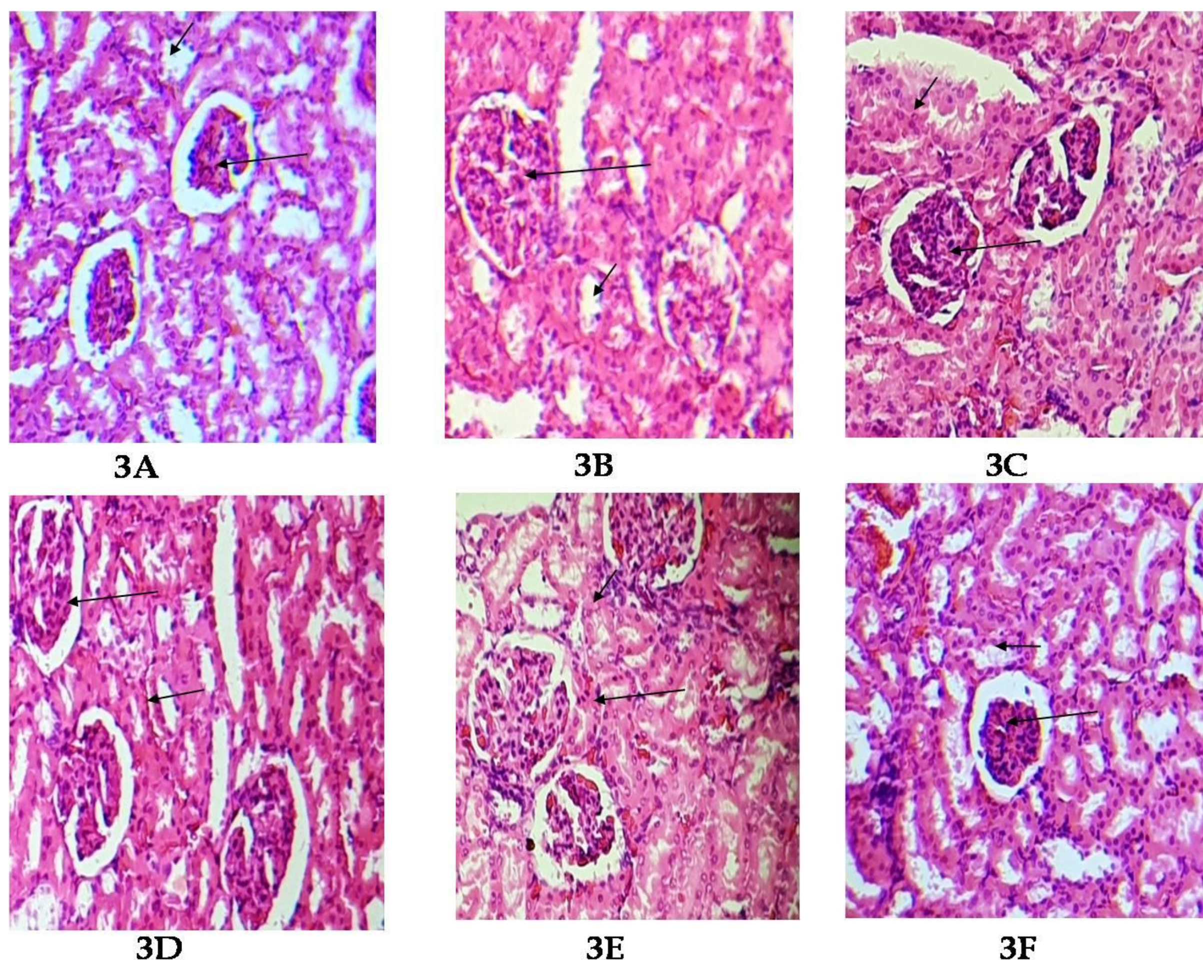

Figure 3: Representative Micrographs of Kidneys of Rats Treated with *L. alata*
Visible renal corpuscle (long arrow) and interstitial space (short arrow) and tubules of control group 3A; 50 mg/kg treatment group (3B); prominent renal corpuscle (long arrow) with mild diffused inflammatory cells in the interstitial (short arrow) with mild tubular necrosis 100 mg/kg treatment group (3C); renal corpuscle (long arrow) and interstitial space (short arrow) and tubules 200 mg/kg treatment group (3D); large renal corpuscle (long arrow) with mild diffused inflammatory cells in the interstitial (short arrow) with mild tubular necrosis 400 mg/kg treatment group (3E); visible atrophied renal corpuscle (long arrow) and interstitial space (short arrow) and tubules.

Phytochemical analysis revealed the presence of phytochemicals similar to those present in the aqueous stem bark extract [12]. Toxicity studies are useful in evaluating safety profile of test compounds and provides valuable information on potential organ damage, pathological and morphological changes which could occur from acute, sub-

acute and chronic usage of such compounds [4].

The oral LD_{50} of *Lophira alata* in mice is high, indicative of relative safety. Increase or decrease in body weights of animals with test compounds could be due to changes in feeding habits or changes in food metabolism [18]. In this study, treatment with *L. alata* did not produce significant changes in body weights of test animals compared to the control showing its inability to negatively affect feeding habit and/or food metabolism.

The organ/weight ratio (OWR) is also useful in providing information on toxicity of test compounds. Increase or decrease in OWR is observed was a sensitive indicator of organ toxicity by known compounds known to be toxicants [19]. No significant increase or decrease was observed in this study.

Changes in haematological parametres in animal toxicity studies is relevant as haematological changes have been demonstrated to have higher translational and concordant values for predicting human toxicity associated with the use of pharmaceuticals [20]. Red blood cell (RBC) count and haemoglobin concentration levels are important for the transportation of respiratory gases while mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, related components of RBC, are useful indices for predicting anaemia [21-23]. Platelet indices viz plateletcrit, platelet count, mean platelet volume and platelet distribution width are important in haemostasis and blood coagulation [24]. In this study, *L. alata* at doses used did not significantly affect haematological and platelet indices.

White blood cells, lymphocytes and monocytes are important in host defence mechanisms, increase in these cell counts indicates activation of the immune system while a decrease corresponds to immunosuppression [25,26]. A decrease in monocytes was observed at higher dose levels (200-800 mg/kg).

The liver is the main organ responsible for metabolism and biotransformation of substances received from the digestive system and hepatic enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) are biomarkers indicative of liver function. These enzymes which increase with acute hepatic infection tend to decrease with prolonged exposure to the toxicants as a result of damage to hepatic cells [27,28]. Increase in serum proteins occur in early stages and may precede observable physiological and behavioural changes in animals [29]. Though treatment with *L. alata* did not significantly affect hepatic enzymes and serum proteins in this study, the highest dose level reduced total and conjugated bilirubin.

The kidneys are responsible for maintaining homeostasis; plasma concentrations of electrolytes, creatinine and urea are useful indicators of nephrotoxicity. Increased levels of urea and creatinine correlate with impaired renal function [30,31], while electrolyte imbalance can be deleterious to the cardiovascular system, central nervous system and the musculature [32,33]. *L alata* at 800 mg/kg decreased creatinine levels but did not significantly affect other renal parameters. The lipid profile is an important health index as lipid abnormalities are risk factors for ischaemic heart diseases and stroke, in particular increased levels of serum total cholesterol, triglycerides and low density lipoprotein cholesterol are associated with coronary heart attack and stroke [34,35]. Though lower doses of *L. alata* used in this study did not significantly affect the lipid profile of the test animals, 800 mg/kg produced an increase in triglycerides.

Histopathological studies involving microscopic examination of tissues is used as a separate tool or in combination with other

diagnostic tests to study the manifestations of disease or for assessing treatment related changes in cell morphology [36,37]. Lower doses of the extract did not produce significant histopathological changes in the brains, livers and kidneys of test animals. The highest dose (800 mg/kg) of the extract caused pyknosis and thickening of white matter of the brain. Consistent with the findings of some renal indices (reduced creatinine levels), 800 mg/kg dose levels caused atrophy of renal corpuscles, tubules and interstitial spaces. Mild fibrosis, fatty changes and visible stenosis was observed 800 mg/kg treatment group, this can be correlated with the increase in triglycerides observed in this group.

Conclusion. Chronic administration of *L. alata* did not adversely affect body weight, haematological indices, lipid profile, liver and kidney function and but showed some histopathological changes at high doses. Lower doses are relatively safe while high doses could be potentially toxic with prolonged administration.

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