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Original Research Article

Studies on the Toxicological Properties of Ethanol Stem-Bark Extract of *Newbouldia laevis (*P. Beauv) Seem in Rats

Adeyinka A. Aderinola[1,2]*, Jane I. Ejiofor[2], Ahmed B. Chindo[2,3]

[1]*Deparment of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago-iwoye, Nigeria.*
[2]*Deparment of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.*
[3]*Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria.*

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ABSTRACT

This research aimed at evaluating the toxicological profile of ethanol stem-bark extract of *N. laevis* in Wistar rats. Repeated oral dose toxicity studies (28 and 90 days) were carried out with 32 rats for 28-day study and 40 rats for 90-day study. The rats were divided into 4 groups (A, B, C and D) of six rats per group and were treated with 250, 500 and 1,000 mg/kg body-weight of the extract for 28 and 90 days respectively. Blood samples were collected at the end of the experiment and were used for Biochemical and Haematological investigations, organs were also collected for histological examination. There was significant ($p \leq 0.05$) reduction in WBCs following 28-day treatment and a significant ($p \leq 0.05$) increase after 90-day treatment. The RBCs, haemoglobin and haematocrit increased significantly ($p \leq 0.05$) at 1,000 mg/kg following 28-day treatment and at all doses for RBCs and haemoglobin after 90-day treatment. A significant ($p \leq 0.05$) increase in HDL and a decrease in LDL was observed after 28-day treatment, the 90 days treatment showed significant ($p \leq 0.05$) decrease in HDL and an increase in LDL. The AST level also increased significantly ($p \leq 0.05$) after 28-day treatment. Histological-examination revealed pathological abnormalities in kidney and liver of the treated rats at all doses. This study showed that extract of *N. laevis* stem-bark was toxic when used at high doses and for a long period of time in the treated rats.

***Keywords*:** Medicinal plants, Toxicological profile, *Newbouldia laevis*, Biochemical and Haematological investigations..

Introduction

The old tradition of using indigenous plant parts for disease remedies and health maintenance irrespective of unknown consequences of such plant is common among people of all continents[1-2] and this is mostly due to the expensive as well as inaccessibility of conventional health care systems and/or the availability of plant medicines.[3-4]

Newbouldia laevis of the Bignoniaceae family, also known as fertility plant and native to tropical Africa is a fast growing evergreen drought tolerant plant that grows up to 7-15 m height. It has large, glossy and deep green leaves with large purple and white flowers.[5] It is a very popular plant in the African folkloric medicine and is highly valued due to its numerous health benefits to humans.

Its various parts are widely used in traditional medicine to treat various diseases including malaria and fever,[6] migraine, skin infection, stomach ache, epilepsy, diabetes, cough, constipation, cardiovascular diseases, urinary tract infection.[7-8]

Extracts from different parts of *Newbouldia leavis* plant have been extensively studied and reported to possess analgesic, anti-nociceptive, anti-inflammatory, uterine contractile, anti-diabetic, anti-cancer, anti-coagulant, antioxidant, antimicrobial, sedative, antimalarial, anti-ulcer, anti-convulsant and depressant activities.[9-17]

*Corresponding author. E mail: aderinola.aderonke@oouagoiwoye.edu.ng
Tel: +2348100407256.

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Plant materials contain active organic principles which in addition to being beneficial could also pose hazardous consequences. Some medicinal plants that were once considered non-toxic have been reported to be hepatotoxic, while some are responsible for renal impairment.[18] It is therefore necessary that plant materials be subjected to scientific evaluation of their toxicological profile in order to establish their safety. Thus, the ethanol stem-bark extract of *Newbouldia leavis* was evaluated for its toxicological implications on some vital organs in Wistar rats.

Materials and Methods

Plant material

The stem-bark of *N. laevis* was collected in March 2014 from Abeokuta, Abeokuta South Local Government Area, Ogun State, Nigeria. The plant was identified and authenticated by Malam Namadi Sunusi of the Department of Botany, Ahmadu Bello University, Zaria and a voucher specimen was deposited in the herbarium section of the Department and voucher number ABU02881 obtained.

Extraction of the plant material

The fresh stem-bark of *N. laevis* was air dried under shade until a constant weight was obtained and then reduced to coarse powder using a grinding machine. Five hundred grams (500 g) of powdered plant material was cold macerated in 4 litres 90% ethanol for 72 hours with intermittent shaking and then filtered with Whatman (No. 3) filter paper. The resultant filtrate was concentrated using a rotatory evaporator maintained at 40°C and then dried over a water bath at 55°C, this was preserved in a levelled air tight container prior to use.

Extract reconstitution

The extract was reconstituted by suspending 3g of crude extract in 10 ml of distilled water to obtain a stock solution of 300 mg/ml.

Drugs and chemicals
All the drugs and chemical reagents used for this study were of analytical grade.

Experimental animals
Wistar rats (120-160 g) of both sexes obtained from the Animal House of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. The animals were kept in clean cages for two weeks' acclimatization and all through the study. The animals were fed with standard rodent pellet diet and water *ad libitum* for the duration of the study and used with strict adherence to the Ethics Guidelines and Research Policy of Ahmadu Bello University, Zaria. Ethical Approval (ABUCAUC/2021/084) was obtained from the ABU Committee on Animal Use and Care.

Toxicity studies
Acute study in mice
The oral median lethal doses (LD_{50}) of the ethanol stem-bark extract of *N. laevis* was determined in mice using method described by.[19] The study was carried out in two phases with a total of 12 mice. In phase 1, three groups of 3 mice each were orally administered with graded doses of 10, 100 and 1,000 mg/kg of the ethanol stem-bark extract of *N. laevis* and observed for signs of toxicity and death in 24 hours. The second phase of the experiment based on the outcome of the first phase, was conducted using doses of 1,600, 2,900 and 5,000 mg/kg of the stem-bark extract in 3 mice and again observed for 24 hours.
The square root of the smallest dose that caused mortality and the highest dose that did not was taken as the estimated median lethal dose (LD_{50}) for the ethanol stem-bark extract of *N. laevis.*

$$LD_{50} = \sqrt{\text{highest non-lethal dose} \times \text{lowest lethal dose}}$$

Repeated dose (28 and 90 days) Oral Toxicity Study in Rats
The repeated dose (28 and 90 days) oral toxicity studies of ethanol stem-bark extract of *N. leavis* was carried out according to Organization for Economic Cooperation and Development (OECD) guidelines number 407 and 408.[20-21] Thirty two (32) and 40 rats of both sexes were randomly divided into 4 treatment groups of 8 rats (4 males and 4 females in separate cages) for 28 days study and 10 rats (5 males and 5 females) for 90 days study per group respectively. Group A, which serve as control were treated with 1 ml/kg distilled water, while rats in groups B, C and D were given graded doses; 250, 500 and 1,000 mg/kg of the extract daily for 28 and 90 days respectively. The rats were allowed free access to feed and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality. The body weight (in gram) of each rat was recorded on day 0 and at weekly intervals throughout the course of the study and the average body weight for the groups was calculated. Each rat was anaesthetized with chloroform and sacrificed a day after 28 or 90 days treatment. Blood samples were collected by cardiac puncture into bottles containing anticoagulant (EDTA and lithium heparin bottles). The blood in EDTA bottles were used for haematological investigation while the blood in lithium heparin bottles were centrifuged at 3500 rpm for 15 min and the plasma collected into clean, dry bottles for biochemical analysis. The liver, kidney, heart, lung, brain, testis and uterus were excised and put in separate bottles and weighed. The relative organ weight ratio of the various organs were calculated as follows[22]:

$$\text{ROW} = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrificed time}} \times 100$$

The isolated organs were then fixed in 10% formalin for histological investigation.

Histopathological investigations
Tissue samples from the selected were fixed in 10% formalin. Slices of tissues measuring about 3-4 µm thickness were cut off and put in an automatic tissue processor and further fixed in 10% formol-saline solution for 2 hours. The selected organs were dehydrated for two hours in each of ascending grades of alcohol (85%, 90% and 100% v/v). The dehydrated tissues were cleared in toluene for two hours and tissue slices were embedded in paraffin wax and left to cool. Blocks were trimmed in microtome at microns and ribbon sections floated in a warm water bath. Suitable sections were then dewaxed in xylene and rehydrated in descending grades of alcohol (100%, 90% and 70% v/v). Sections were then stained in haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in Scott's tap water and stained in eosin for 3 minutes. The sections were then rinsed and dehydrated in ascending grades of alcohol (70%, 90% and 100% v/v). The sections were cleared in xylene and mounted in a box. They were examined microscopically for pathological lesions, photomicrographs of representative lesions were taken at × 400 magnifications as described by Bancroft and Gambe, 2008.[23]

Estimation of Biochemical parameters
Effects of ethanol stem-bark extract of *N. laevis* were evaluated by assessing the alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), serum albumin, creatinine, urea, chloride, sodium, potassium, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels using standard diagnostic test kits (Randox Laboratories, Crumlin, UK) on Automated Clinical System (Sychron Clinical System, model: CX5PRO; Beckman Coulter Inc., Galway, Ireland) as previously described.[24-25]

Evaluation of Haematological parameters
The effect of ethanol stem-bark extract of *N. laevis* were evaluated on packed cell volume (PCV), haemoglobin concentration (Hb or Hgb), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), white blood cell (WBC) count and white blood cell differential count as described [26] using an automated haematological machine.[26]

Statistical analysis
The statistical analysis of the data was done using Graph Prism version 6.00. Differences in mean between the groups were determined using One Way Analysis of Variance (ANOVA), followed by Dunnett Multiple Comparison Test. The results obtained were expressed as mean ± standard error of mean (SEM), differences were considered significant at $p \leq 0.05$.

Results and Discussion

Newbouldia laevis is a plant used traditionally for treatment of several illnesses and diseases inspite of unknown toxic effects. This study investigated the safety profile of ethanol stem-bark extract of this plant in rats and mice.
In the oral acute toxiciy study, there was no observable signs of behavioural changes nor death seen with up to 5,000 mg/kg dose of ethanol stem-bark extract of *N. laevis* in mice (Table 1). The oral median lethal doses (LD_{50}) of *N. laevis* stem-bark extract were estimated to be greater than 5000 mg/kg suggesting that the extract was relatively safe and non-toxic.[19] This result is consistent with the report of [27-28] who reported that no mortality was recorded up to 5000 mg/kg in the root and ethanol leaf extracts of *N. laevis* and the estimated LD_{50} was greater than 5000 mg/kg.
For the 28 days treatment, an obvious increase in weight was seen as from week 2 at which the group that received the highest dose (1,000 mg/kg) showed significant ($p \leq 0.05$) increase in weight compared to all other groups, but subsequently in weeks 3 and 4, significant ($p \leq 0.05$) and dose dependent weight increase was observed at all doses administered (Figure 1).
In the 90 days treatment, slight but insignificant ($p \geq 0.05$) weight gain was seen in week 1. Continuous increase in weight was observed as from week 2, but significant ($p \leq 0.05$) only for the 1,000 mg/kg group. In weeks 3 and 4, the weight increase was significant ($P \leq 0.05$) for the 500 mg/kg and 1,000 mg/kg groups and significant ($p \leq 0.05$) subsequently for all the groups from weeks 5 to 12 and somewhat in a dose dependent manner (Figure 2).
Changes in body weight is considered a sensitive indicator of general health status and/or indicator of adverse effect of drugs and chemicals.[29] The dose dependent increase in body weight observed in the 28 and 90 days toxicity study, suggested that the normal food intake and/or metabolic processes necessary for growth and development were not

altered in the rats, rather the crude extract improved their appetite and as a result they were growing. Similar increase was also reported in body weight of rats treated with *N. laevis* leaf extract for 28 days.[30]

The relative weights of liver, heart, kidney, testes, spleen and lungs were reduced at 250 mg/kg in the 28 days treatment compared to the control group, which were significant ($p \leq 0.05$) for the heart, testes and spleen. There were no significant ($p \geq 0.05$) changes in weights of the organs at 500 mg/kg in the 28 days test. At 1,000 mg/kg 28 days treatment, the weights of the heart, brain and spleen were reduced, while the others increased, but none were significant ($p \geq 0.05$).

All the organs showed increase in weight at 1,000mg/kg in the 90 days treatment, which was significant ($p \leq 0.05$) for the liver, pancreas, spleen and lungs. Changes in weights were not significant ($p \geq 0.05$) at 250 and 500 mg/kg compared to the control (Table 2).

Organ weight is another important index of physiological and pathological status; and the organ weights relative to body weights is often the more viable and sensitive index or marker of toxicity than the absolute weights.[31-32] The changes in the relative organ weight observed in this study is an indication that the crude extract did not alter the organs of functionality in the treated rats. The reasons for the significant reduction in weights of heart, testes and spleen in the 28 days treatment is not known, but may be related to immune response of phargocytic cells to the presence of exogenous agents.

Table 1: LD_{50} Determinations of Ethanol Crude Extract and Fractions of *Newbouldia laevis* Stem-bark in Mice and Rats

Phase I		
No. of Mice/Rats	**Dose (mg/kg)**	**Mortality**
3	1000	None
3	100	None
3	10	None
Phase II		
No. of Mice/Rats	Dose (mg/kg)	Mortality
1	1600	None
1	2900	None
1	5000	None

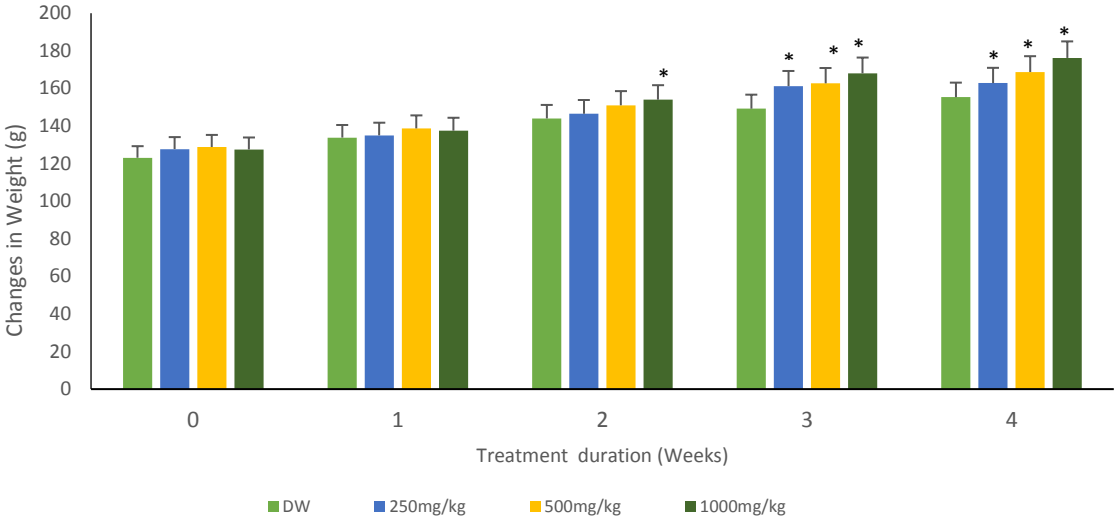

Figure 1: Changes in body weight following 28 days' oral administration of ethanol stem-bark extract of *Newbouldia laevis* in rats
n = 8; Values are Mean ± SEM; Statistics: repeated measure ANOVA and Bonferroni's post hoc test at *$p \leq 0.05$ compared to control group.

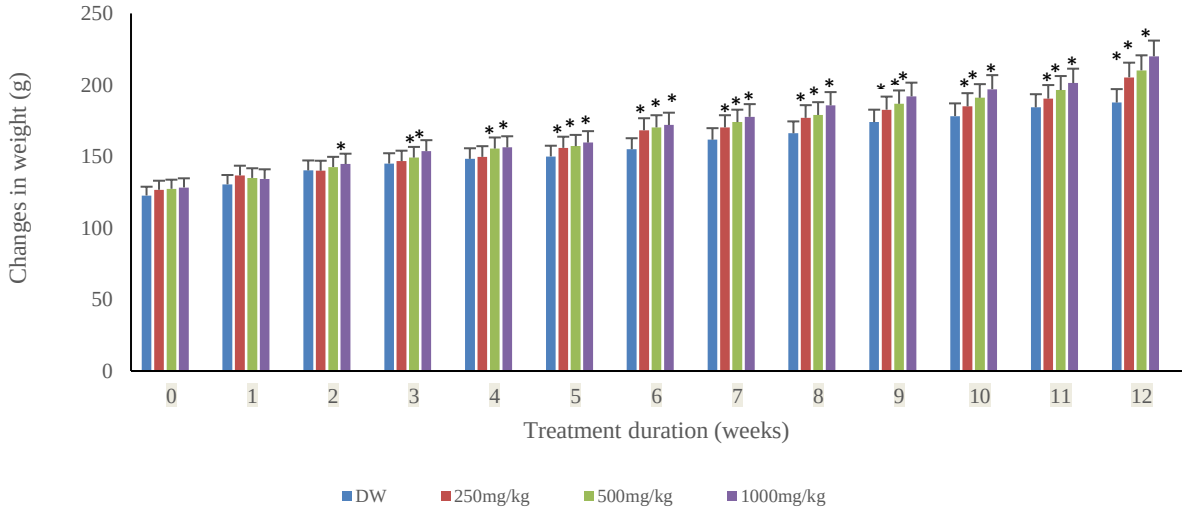

Figure 2: Changes in body weight following 90 days' oral administration of ethanol stem bark extract of *Newbouldia laevis* in rats
n = 10; Values are Mean ± SEM; Statistics: repeated measure ANOVA and Bonferroni's post hoc test at *$p \leq 0.05$ compared to control group.

However, the increase in relative organ weights seen in the 90 days toxicity study may be related to the effect of the crude extract on the organs and may also be as a result of histological changes seen in them. The 28 days treatment showed reduced white blood cells count at all doses of the extract, which was significant ($p \leq 0.05$) at 500 and 1,000 mg/kg doses. The red blood cells count, haemoglobin, PCV, MCHC and lymphocyte were significantly ($p \leq 0.05$) increased at 1,000 mg/kg, all were insignificantly ($p \geq 0.05$) reduced at the lower doses after 28 days treatment, while neutrophil count was reduced significantly ($p \leq 0.05$). Platelet and MCV levels increased insignificantly ($p \geq 0.05$) at all doses. In the 90 days treatment, an increase in white blood cells count at all doses was observed, which was significant at lower doses. There were increase in red blood cells count and haemoglobin at all dose levels which were significant ($p \leq 0.05$) at 500 mg/kg. PCV was significant ($p \leq 0.05$) at 250 mg/kg. There was a consistent reduction in lymphocyte level at 250 mg/kg in both 28 and 90 treatments, which was significant in the 90 days treatment. However, the increase in platelet count in the 90 days treatment was significant ($p \leq 0.05$) only at 250 mg/kg (Table 3).

WBCs are the normal immune components of the body system that scavenge foreign agents and mop up cellular debris and pathogenic invaders (bacteria & viruses)[33] and which tend to increase in the presence of invaders and in this case, the presence of the extract. Increase in WBC count in the 90 days treatment may be related to normal immunological reaction of the rats to foreign agents. Lymphocyte count increased at higher doses of the crude extract, the increase could be the usual immune response to encountered exogenous agents, as significant lymphocyte count increase is only seen during inflammation of body organs especially the liver,[34] this may have contributed to cytoplasmic vacuolation seen in the liver histology at higher doses of the crude extract.

Hematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health. They help to determine the extent of the deleterious effect of foreign compounds including plant extract on the blood parameters of animals.[35] The increase in red blood cell indices (iron, Hbg, MCHC) seen in this study may suggests presence of haematinic or red blood cell build up potentials in the crude extract; the blood building up effect of the crude extract may have help in stimulation of erythropoietin release from the kidney for blood synthesis or enhanced oxygen carrying capacity and/or improved bone marrow functions. The increase in red blood cell parameters seen in this study may be the reason for the traditional use of this plant in pregnancy. Low Hct usually seen as reduced circulating blood cell mass (including PCV, Hbg, and red cell count) results in iron deficiency anaemia. Thus, the increase in Hct seen with the crude extract suggests that the crude extract may have the ability to prevent anaemia. This finding is in concordance with reports[30] who reported that oral administration of *N. laevis* leaf extract for 28 days also caused increase in haematological parameters of the treated rats.

The 1,000 mg/kg dose in the 28 days treatment showed significant ($p \leq 0.05$) increase in HDL concentration and insignificant increase at lower doses (250 and 500 mg/kg). LDL was dose dependently decreased in the 28 days treatment, which was significant at 500 and 1,000 mg/kg. The total cholesterol was reduced at 250 and 500 mg/kg in the 28 days treatment, which was significant ($p \leq 0.05$) at 250 mg/kg as well as triglyceride. An increased in total cholesterol was observed at 1,000 mg/kg in 28 days treatment.

The concentration of HDL was dose dependently reduced in the 90 days treatment and was significant ($p \leq 0.05$) at 1,000 mg/kg. LDL significantly ($p \leq 0.05$) increase in the 90 days treatment, but reduced remarkably at 250 mg/kg. An increased in total cholesterol was observed at all the 3 doses in the 90 days treatment as triglyceride, which was significant ($p \leq 0.05$) at both 500 and 1000 mg/kg for both indices (Table 4).

Lipid profile evaluation provides useful information on lipid metabolism status and predisposition of animals to cardiovascular diseases. A rise in lipid profile particularly the LDL is usually an indication of coronary artery events including atherosclerosis which are common causes of mortality and morbidity.[36] Reduction in the LDL level produced by the crude extract suggests that the extract might not be harmful to the cardiovascular system. A significant increase in the levels of LDL, total cholesterol and triglycerides with a decrease HDL level was observed after 90 days treatment with the crude extract. This finding suggests that long term use of this extract especially at higher doses may not be advocated because of the build-up of low density lipoprotein that are implicated in cardiovascular diseases.[37] Increase in triglycerides causes liver inflammation and may have contributed to the changes seen on liver histology at higher doses of the crude extract. Overall, the observed effects of the crude extract on the serum lipid indices suggest that the extract may possess hypolipidemic potentials when used for short durations at high doses or for longer durations at low doses. The presence of saponins in the extract as one of its phytochemicals may be responsible for this effect as saponins have been reported to possess the ability to bind onto cholesterol moieties to inhibit their absorption and/or mop them up.[37] High density lipoproteins are known to demobilise free fatty acids off the walls of blood vessels for onward excretion.[38]

The 28 days treatment showed increase in creatinine level at all doses which was significant ($p \leq 0.05$) at 1,000 mg/kg. Urea increased at all doses in the 28 days treatment. The chloride level also increased in the 28 days treatment and was significant at 500 mg/kg.

Table 2: Relative Organ Weight Changes Following 28 and 90 days Oral Administration of Ethanol Stem-bark Extract of *Newbouldia laevis* in Rats

Parameters investigated	Treatment groups (mg/kg)							
	28 days				90 days			
	Distilled H_2O (1 ml/kg)	ethanol crude extract 250	500	1000	Distilled H_2O (1ml/kg)	ethanol crude extract) 250	500	1000
Liver	4.96±0.24	4.72 ± 0.32	4.98 ± 0.56	4.99 ± 0.52	4.55 ± 0.32	5.07 ± 0.37	5.18 ± 0.68	5.63 ± 0.03*
Heart	0.72±0.05	0.51 ± 0.02*	0.56 ± 0.04	0.60 ± 0.03	0.50 ± 0.03	0.52 ± 0.02	0.60 ± 0.06	0.70 ± 0.01
Kidney	0.98±0.05	0.95 ± 0.06	1.00 ± 0.03	0.98± 0.05	0.98 ± 0.07	0.97 ± 0.26	1.16 ± 0.09	1.23 ± 0.03
Testes	3.92±0.45	2.59 ± 0.15*	3.86 ± 0.15	4.13 ± 0.33	3.95 ± 0.15	4.13 ± 0.14	4.30 ± 0.40	4.40 ± 0.20
Brain	1.49±0.09	1.56 ± 0.04	1.60 ± 0.03	1.06 ± 0.05	1.47 ± 0.08	1.56 ± 0.07	1.57 ± 0.07	1.57 ± 0.06
Pancreas	0.35±0.03	0.36 ± 0.04	0.41 ± 0.08	0.42 ± 0.08	0.33 ± 0.05	0.33 ± 0.09	0.33 ± 0.03	0.48 ± 0.05*
Spleen	0.59±0.04	0.38 ± 0.03*	0.51 ± 0.04	0.58 ± 0.08	0.55 ± 0.11	0.40 ± 0.12	0.44 ± 0.13	0.80± 0.15*
Uterus	0.89±0.20	1.19 ± 0.24	1.33 ± 0.22	1.40 ± 0.15	0.77 ± 0.12	1.00 ± 0.01	1.05 ± 0.65	1.07 ± 0.35
Lungs	1.02±0.05	0.93 ± 0.11	1.05 ± 0.03	1.05 ± 0.04	1.17 ± 0.09	1.10 ± 0.12	1.17 ± 0.07	1.34 ± 0.09*

n = 8; Values are Mean ± SEM, Statistics: One Way ANOVA followed by Dunnet's Post hoc test at *$p \leq 0.05$ compared to compared to control group

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Table 3: Changes in Haematological Parameters following 28 and 90 days OralAdministration of Ethanol Stem-bark Extract of *Newbouldia laevis* in Rats

Parameters investigated	Treatment groups (mg/kg)							
		28 days ethanol crude extract				90 days ethanol crude extract		
	Distilled H_2O (1 ml/kg)	250	500	1000	Distilled H_2O (1ml/kg)	250	500	1000
WBC × 10^3	8.92 ± 0.94	8.17 ± 0.88	4.95 ± 1.11*	6.27 ± 0.69*	4.96 ± 0.48	8.22 ± 1.20*	8.28 ± 2.08*	5.94 ± 1.60
RBC × 10^6	7.90 ± 0.21	7.40 ± 0.65	7.38 ± 0.36	8.83 ± 0.36*	8.41 ± 0.22	8.93 ± 0.46	9.36 ± 0.38*	8.80 ± 0.31
Haemoglobin (g/dl)	12.98 ± 0.37	12.25 ± 1.15	12.33 ± 0.64	15.08 ± 0.72*	14.72 ± 0.32	14.80 ± 0.85	16.16 ± 0.41*	15.28 ± 0.66
Haematocrit (PCV %)	47.38 ± 1.19	45.30 ± 4.02	46.08± 2.60	53.18 ± 2.83*	45.90 ± 0.92	49.50 ± 0.87*	48.18 ± 1.38	46.57 ± 2.55
Platelet × 10^3	661.7 ± 101.1	811.8 ± 66.26	815.5 ± 57.56	805.0 ± 21.7	779.2 ± 68.56	857.7 ± 47.80*	681.4 ± 60.27	741.7 ± 187.8
Neutrophil (%)	23.67 ± 3.47	22.08 ± 1.91	17.07 ± 4.54*	19.85 ± 3.52*	13.62 ± 3.61	32.43 ± 2.12*	12.86 ± 2.23	6.53 ± 2.61
Lymphocyte (%)	55.94 ± 1.60	54.85 ± 9.42	59.9 ± 3.06	64.55 ± 4.45*	63.48 ± 3.32	52.77 ± 1.60*	63.98 ± 3.73	63.83 ± 8.76
MCHC (g/dL)	27.38 ± 0.24	26.95 ± 0.73	26.82 ± 0.30	28.40 ± 0.29	32.07 ± 1.51	29.90±0.88	33.54±2.11	32.81 ± 1.45
MCV (Fl)	60.00 ± 0.70	61.27 ± 0.27	62.33 ± 0.64	60.08 ± 1.36	54.58 ± 2.06	55.43±1.73	51.47±1.94	52.92 ± 2.22

n = 8; Values are Mean ± SEM, Statistics: One Way ANOVA followed by Dunnet's Post hoc test at *$p \leq 0.05$ compared to control group.
Key: WBC = white blood cell; RBC = red blood cell; HCT = Packed Cell Volume; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume

Table 4: Changes in Serum Lipid Indices following 28 and 90 days Oral Administration of Ethanol Stem- bark Extract of *Newbouldia laevis* in Rats

Parameters investigated	Treatment groups (mg/kg)							
		28 days ethanol crude extract				90 days ethanol crude extract		
	Distilled H_2O (1 ml/kg)	250	500	1000	Distilled H_2O (1ml/kg)	250	500	1000
High density lipoprotein -HDL (nmol/L)	37.33 ± 4.16	37.83 ± 2.75	38.33 ± 3.31	50.67 ± 5.68*	26.67 ± 1.98	26.00 ± 3.51	24.40 ± 0.87	20.67±4.84*
Low density lipoprotein - LDL (nmol/L)	18.83± 2.26	14.17 ± 4.95	12.33 ± 0.84*	9.33 ± 1.54*	12.17 ± 1.25	14.33 ± 2.60	19.60 ± 2.21*	21.33± 2.91*
Total Cholesterol (nmol/L)	67.67± 6.15	54.33 ± 3.33*	61.00 ± 3.65	69.33 ± 6.00	44.33 ± 2.65	47.00 ± 6.43	53.40 ± 3.42*	50.00 ± 5.51*
Triglyceride -TG (nmol/L)	42.83 ±10.26	30.83 ± 4.25*	43.00 ± 5.14	47.17 ± 5.88	27.33 ± 3.59	32.33 ± 6.33	45.40 ± 4.68*	40.67±2.96*

n = 8; Values are Mean ± SEM, Statistics: One Way ANOVA followed by Dunnet's Post hoc test at *$p \leq 0.05$ compared to control group

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Table 5: Changes in Renal Function Indices following 28 and 90 days Oral Administration of Ethanol Stem-bark Extract of *Newbouldia laevis* in Rats

Parameters investigated	Treatment groups (mg/kg)							
	28 days				90 days			
	Distilled H_2O (1ml/kg)	ethanol crude extract			Distilled H_2O (1ml/kg)	ethanol crude extract)		
		250	500	1000		250	500	1000
Creatinine (µmol/L)	0.31 ± 0.03	0.38 ± 0.67	0.35 ± 0.06	0.43 ± 0.04*	0.58 ± 0.03	0.66 ± 0.07*	0.59 ± 0.04	0.57 ± 0.03
Urea (µmol/L)	8.83 ± 0.87	11.17 ± 1.64	9.67 ± 1.36	11.17 ± 1.11	33.83 ± 1.58	37.60 ± 4.40*	31.33 ± 2.73	31.33 ± 1.76
Na+	141.7 ± 0.84	143.5 ± 1.23	143.7 ± 1.61	144.5 ± 1.71	144.2 ± 0.48	146.3 ± 2.03	146.4 ± 1.17	144.7 ± 0.88
K+	4.40 ± 0.20	4.32 ± 0.09	4.33 ± 0.16	4.42 ± 0.08	5.03 ± 0.03	5.00 ± 0.15	4.97 ± 0.9	4.88 ± 0.12
Cl^-	96.83 ± 1.17	98.00 ± 1.21	102.3 ± 2.03*	100.5 ± 1.78	98.63 ± 0.29	98.90 ± 1.00	98.53 ± 0.67	98.17± 0.50

n = 8; Values are Mean ± SEM, Statistics: One Way ANOVA followed by Dunnet's Post hoc test at $*p \leq 0.05$ compared to control group

Table 6: Changes in Liver Function Indices following 28 and 90 days Oral Treatment with Ethanol Stem-bark Extract of *Newbouldia laevis* in Rats

Parameters investigated	Treatment groups (mg/kg)							
	28 days				90 days			
	Distilled H_2O (1ml/kg)	ethanol crude extract			Distilled H_2O (1ml/kg)	ethanol crude extract)		
		250	500	1000		250	500	1000
Conjugated bilirubin(µmol/L)	0.12 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
Total bilirubin (µmol/L)	0.28 ± 0.03	0.37 ± 0.05	0.37 ± 0.02	0.43 ± 0.33*	0.25 ± 0.03	0.33 ± 0.09	0.32 ± 0.04	0.30 ± 0.06
Albumin (g/L)	2.75 ± 0.27	2.82 ± 0.15	3.32 ± 0.15	3.65 ± 0.13	4.13 ± 0.20	4.17 ± 0.19	3.92 ± 0.30	3.60 ± 0.36
Total protein (g/L)	5.32 ± 0.56	5.55 ± 0.32	6.35 ± 0.39	6.98 ± 0.16	10.02 ± 0.22	10.27 ± 0.15	9.82 ± 1.02	8.80 ± 1.11*
ALP (µ/L)	61.47 ± 2.77	55.92 ± 4.09*	56.32 ± 3.91	60.75 ± 7.66	60.88 ± 2.09	65.13 ± 4.85*	61.90 ± 3.78	61.9 ± 4.09
AST (µ/L)	49.67 ±1.50	53.92 ± 3.61	44.50 ± 2.25	58.42 ± 3.67*	53.95 ± 2.78	57.13 ± 4.99*	53.90 ± 4.66	53.2 ± 4.34
ALT (µ/L)	23.58 ± 2.12	22.13 ± 1.60	18.38 ± 0.41	20.03 ± 1.16	19.83 ± 0.95	21.20 ± 2.04	19.82 ± 2.14	19.70 ± 1.23

n = 8; Values are Mean ± SEM, Statistics: One Way ANOVA followed by Dunnet's Post hoc test at $*p \leq 0.05$ compared to control group
Key: ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase

An insignificant ($p \geq 0.05$) increase in creatinine at 250 and 500 mg/kg doses was observed in the 90 days treatment, with the 1,000 mg/kg dose level being slightly reduced. Urea level increased significantly ($p \leq 0.05$) at 250 mg/kg in the 90 days treatment with slight reduction at higher doses (500 and 1,000mg/kg). The potassium and sodium level increased slightly in both tests, but none was significant ($p \geq 0.05$). Change in chloride level in the 90 days treatment was insignificant ($p \geq 0.05$) (Table 5).

The increase in both creatinine and urea following 28 and 90 days administration of the crude extract suggested kidney related damaging effects particularly at high doses, given that urea and creatinine are waste products of protein metabolism excreted in the kidney and are used as important markers of renal dysfunction. The rise in serum creatinine concentrations forms the basis of diagnosis of chronic kidney disease including renal damage or tubular necrosis [25]. The increase in creatinine and urea in this study agrees with the previous study by.[28] The kidney histology in this study also showed tubular necrosis and glomerular necrosis with lymphocyte hyperplasia that might have caused this increase in creatinine and urea and increase in systemic lymphocyte seen in this study.

Sodium (Na^+) and Cl^- ions are usually the predominant cation and anion of the extracellular fluid (ECF). Na^+ is largely associated with Cl^-, available as NaCl in the regulation of acid-base equilibrium.[39] Only significant alteration in the extracellular fluid (ECF) ionic concentration of electrolytes poses life-threatening toxicity, given that functional kidneys often make necessary physiological adjustment that effectively maintains body fluid equilibrium, thus the minimal increase seen in both ions in this study could be due to the observed histological changes in kidneys of the crude extract treated rats. The changes in potassium level was insignificant and potassium as other electrolytes is a micronutrient available in minute amount for optimal functioning of the body system. Depletion or increase in potassium level that causes shift in the basal level often results in deviation in the steady state ionic concentration of the body fluid.[39]

Slight increase in conjugated bilirubin level was observed at all doses in both tests as do the total bilirubin, which was significant ($p \leq 0.05$) at 1,000 mg/kg 28 days treatment. Albumin level increased dose dependently in the 28 days treatment, but was not significant. The total protein level showed dose dependent and insignificant ($p \geq 0.05$) increase at all doses in the 28 days treatment. The ALP level reduced at all doses in the 28 days treatment while AST level increased significantly ($p \leq 0.05$) at 1,000 mg/kg in the 28 days treatment.

In the 90 days treatment, albumin level insignificantly ($p \geq 0.05$) increased at 250 mg/kg, with reduction at 500 and 1,000 mg/kg doses that was not significant. The total protein level reduced in the 90 days treatment at higher doses, which was significant ($p \leq 0.05$) at 1,000 mg/kg. The ALP level significantly ($p \leq 0.05$) increased in the 90 days treatment, AST level increased significantly ($p \leq 0.05$) at 250 mg/kg in the 90 days treatments, while the slight changes in ALT level was insignificant ($p \geq 0.05$) in both tests (Table 6).

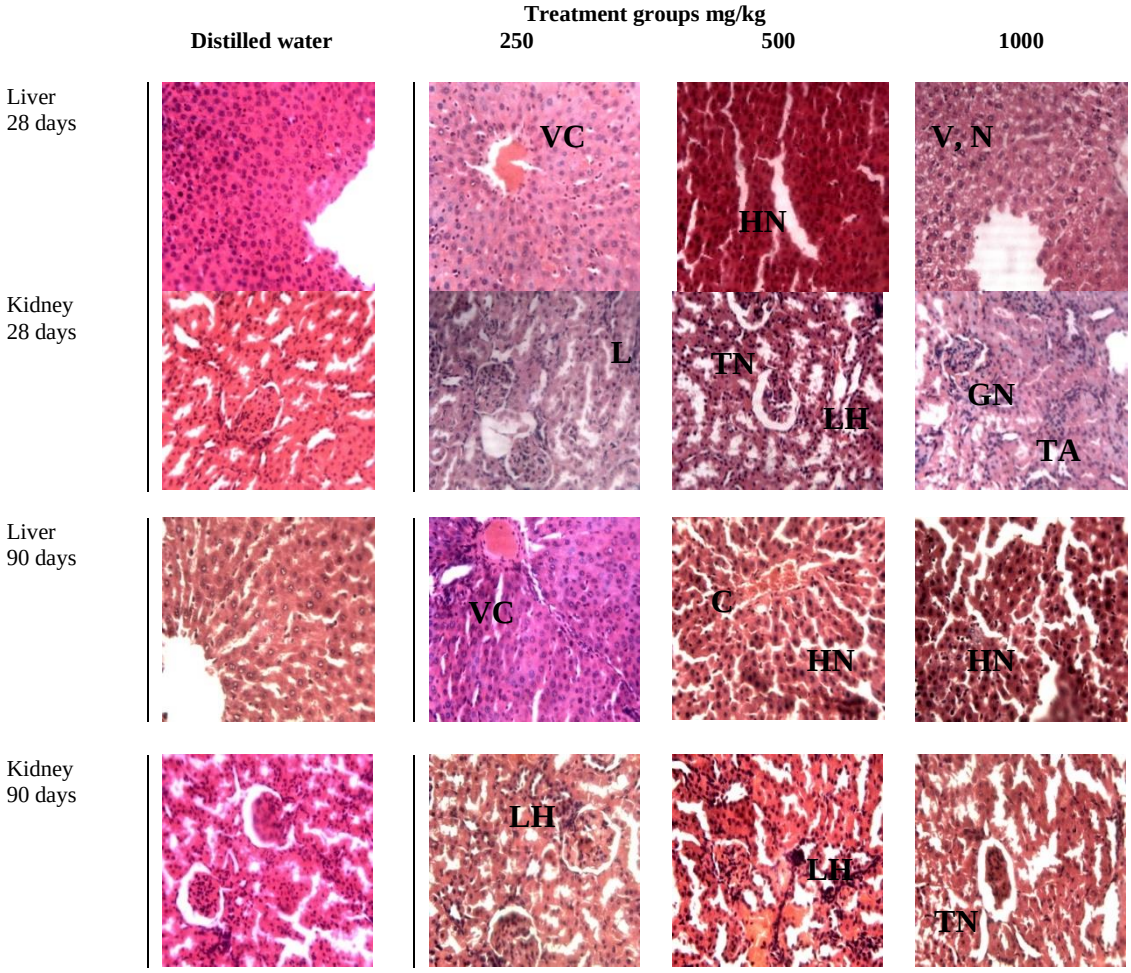

Figure 3: Liver and Kidney sections of rats treated with *N. leavis* for 28 and 90 days.

The result obtained showed pathological changes that progressed from slight lesions in 28 days treatment to moderate lesions in the 90 days treatment compared to normal architecture of control. There were no gross pathological changes in brain, heart and uterus at any of the extract doses in both 28 and 90 days treatments. Venous congestion at 250mg/kg, slight hepatocellular necrosis at 500 mg/kg and cytoplasmic vacuolation with necrosis at 1,000 mg/kg was observed in liver in both 28 and 90 days treatment with ethanol crude extract of *Newbouldia laevis* stem-bark compared to the distilled water group. Lymphocyte hyperplasia at 250 mg/kg, tubular necrosis with slight lymphocyte hyperplasia at 500 mg/kg, and tubular adhesion with slight glomerular necrosis at 1,000 mg/kg was observed in kidney liver in both 28 and 90 days treatment with ethanol crude extract of *Newbouldia laevis* stem-bark compared to the distilled water group

The liver maintains metabolic functions and detoxification of exogenous and endogenous substances and also excretes waste products through the bile. Liver damage may be in form of changes in serum levels of many biochemical markers like AST, ALT, bilirubin, total protein, albumin and alkaline phosphatase as investigated in this study.[40] Elevated levels of liver enzymes or other markers are often diagnostic of underlying cellular injuries. The total bilirubin significantly increased at higher dose of the crude extract and this may suggests a liver function derangement that diminished the ability of the hepatocytes to conjugate bilirubin. Decrease in total protein and albumin levels usually occur in extensive liver damage. Albumin is produced exclusively by the liver and therefore decrease in its synthesis is an indication of liver injury and its increase is often associated with acute infections or stress. Increase in bilirubin, albumin and total protein observed in this study was in line with the report by[30] who also reported similar increase in the parameters after 28 days treatment with *N. laevis* leaf extract.

The reduced and increased levels of ALP in this study are indicative of abnormal conditions. The reduction may probably be related to physiological conditions, but the increase has been reported as a marker of obstructive jaundice or cholestasis.[41] The insignificant effect at higher doses may be due to tubular adhesion seen in the histology of the kidney. Unconjugated bilirubin often results in hepatocellular damage and hepatic biliary tract obstruction[41] which partly may have caused the hepatocyte necrosis found in liver. The pattern of increase in aspartate aminotransferase (AST) level suggests both dose and duration of treatment related effect which may be an indication of more deleterious effect at prolonged treatment or with higher doses. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood or plasma had been reported as markers or indication of hepatocellular injury, but ALT is a more specific liver damage marker than AST which also indicate damage at other tissues where it is also found, such as the heart[42] and also leaks out of the hepatocytes into the blood during peroxidative liver membrane damage that causes increase in membrane permeability.[43]

Conclusion

In conclusion, this study showed that the ethanol stem-bark extract of *N. laevis* was toxic when used at high doses and for a long period of time in rats treated for 28 and 90 days.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Yang M, Zihuan W, Yudan W, Guoyin K, Guy SSN, Shengbao C. 'Acute and sub-acute toxicity evaluation of ethanol extract from aerial parts of *Epigynum auritum* in mice. Food Chem Toxicol. 2019; 131(1):10534. https://doi.org/10.1016/j.fct.2019.05.042.
2. Dawang DN, Mashat MV, Nyam DB, Pam PG, Tongwring DP. Ethnobotanical studies of medicinal plants in Barkin Ladi Local Government Area, Plateau State, Nigeria. IOSR J Pharm Biol Sci. 2016; 11(1):18-22.
3. Ahn K. The Worldwide Trend of using Botanical Drugs and Strategies for Developing Global Drugs. BMB Rep. 2017; 50(3):111-116.
4. Josephine Ozioma EO, Nwamaka Chinwe OA. Herbal medicines in African traditional medicine. In: Builders PF, editor. Herbal Medicine. London: IntechOpen. 2019; 191-214. DOI:10.5772/intechopen.80348.
5. Umeokoli BO, Ekeh MN, Eze P, Umeyor C, Abba C. Improved Gastric Lesion of Ulcerogenic Mice treated with Bark Extract and Fractions of *Newbouldia laevis*. Afr J Pharm Pharmacol. 2015; 9(21):553-560. https://doi.org/10.5897/AJPP2015. 4331.
6. Agbodeka K, Gbekley HE, Karou SD, Anani K, Agbonon A, Tchacondo T, Batawila K, Simpore J, Gbeassor M. Ethnobotanical Study of Medicinal Plants Used for the Treatment of Malaria in the Plateau Region, Togo. Pharm Res. 2016; 8:S12-18.
7. Amujoyegbe OO, Idu M, Agbedahunsi JM, Erhabor JO. Ethnomedicinal Survey of Medicinal Plants Used in the Management of Sickle cell Disorder in Southern Nigeria. J Ethnopharmacol. 2016; 185:347-60. doi: 10.1016/j.jep.2016.03.042.
8. Appiah KS, Oppong CP, Mardani HK, Omari RA, Kpabitey S, Amoatey CA, OnwonaAgyeman S, Oikawa Y, Katsura K, Fujii Y. Medicinal Plants Used in the Ejisu-Juaben Municipality, Southern Ghana: An Ethnobotanical study. Med. 2019; 6:1. doi:10.3390/medicines6010001.
9. Ukwuani-Kwaja AN, Adikwu JE, Abdullahi A. Acute oral toxicity and antipyretic activity of methanolic leaves extract of Newbouldia laevis and Securidica lungipedunculata in albino rats. Asian J Biochem Pharm Res. 2018; 8(1):67-72. [DOI:10.24214/AJBPR/8/1/6772].
10. Bosha JA, Sule S, Asuzu IU. Antidiabetic and Hypolipidemic Effects of Methanol Leaf Extract of *Newbouldia laevis* in Alloxan Induced Diabetic Rats. Adv Res Gastroentero Hepatol. 2019; 13(3): 555863.
11. Akande EB, Oladejo BO, Oladunmoye MK, Abodunrin TF. Antibacterial Effects of *Newbouldia laevis* {P. BEAUV} on the Bacteria Isolated from the Blood of Hepatitis C Virus Positive Individuals. APRJ. 2020; 4(1): 26-34. DOI: 10.9734/aprj/2020/v4i130077.
12. Ndarubu TA, Jigam AA, Olufunmilola AH, Famous OI, Damola AS. Acute Toxicity Studies and Anti-plasmodial Potentials of *Newbouldia laevis* and *Crateva adansonii* in *Plasmodium Berghei*-infected Mice. Iran J Toxicol. 2020; 14(2):93-104.
13. Salawu MO, Raji NH, Oloyede HB. Antimalarial Activity of Aqueous Stem Bark Extract of *Newbouldia laevis* in *Plasmodium berghei*-Infected Mice. Centrepoint J (Science Edition). 2020; 26(1): 14-36. http//www.unilorin.edu.ng/centrepoint. CPJ 2020002/26102.
14. Habu JB, Ibeh BO. *In vitro* Antioxidant Capacity and Free Radical Scavenging Evaluation of Active Metabolite Constituents of *Newbouldia laevis* Ethanolic Leaf Extract. Biol Res. 2015; 48(1):16. https://doi.org/10.1186/s40659-015-0007-x.
15. Arnold CI, Emmanuel EA, Amarachi PE. Antiplasmodial Activity of the Ethanol Leaf Extract of *Newbouldia laevis.* World J Pharm Sci. 2017; 6(4):50-61. doi: 10.20959/Wjpps201704-8716.
16. Igboasoiyi AC, Attih EE, Egeolu AP. Antiplasmodial Activity of the Ethanol Leaf Extract of *Newbouldia laevis*. World J Pharm Sci. 2017; 6(4):50-61.
17. Murtala AA, Akindele AJ. Anxiolytic- and Antidepressants-like Activities of Hydroethanol Leaf Extract of *Newbouldia leaves* (P. Beauv.) Seem. (Bignoniaceae) in Mice. J Ethanopharmacol. 2020; 249:112420. Doi:10.1016/j.jep.2019.112420.
18. Shafaei A, Farsi BMK, Ahamed MJA, Siddiqui IH, Attitalla I Zhari, Asmawi MZ. Evaluation of Toxicological and Standardization Parameters and Phytochemical Investigation

of *Ficus deltoidea* Leaves. Am J Biochem Mol Biol. 2011; 1:237-243.
19. Lorke D. A New Approach to Practical Acute Toxicity Testing. Arch Toxicol. 1983; 54(4):275-87. PMID: 6667118. doi: 10.1007/BF01234480.
20. OECD (Organization for Economic Cooperation & Development). OECD Guidelines for the Testing of Chemicals. Test Guideline 407. 1995; Repeated Dose 28-day Oral Toxicity Study in Rodents.
21. OECD (Organization for Economic Cooperation & Development). OECD Guidelines for the Testing of Chemicals. Test Guideline 408. 1998; Repeated Dose 90-day Oral Toxicity Study in Rodents.
22. Lakmichi H, Bakhtaoui FZ, Chemseddoha AG, Ezoubeiri A, Kenza L. "Toxicity Profile of the Aqueous Ethanol Root Extract of *Corrigiola telephiifolia Pourr. (Caryophyllaceae)* in Rodents". Evid Based Complementary Altern Med. 2011; 317090, 10 pages. https://doi.org/10.1155/2011/317090.
23. Bancroft J, Gambe M. *Theory and Practice of Histological Techniques*. 6th ed. Philadelphia: Churchill Livingston Elsevier. 2008; pp 725.
24. Roy AV. Rapid Method for Determining Alkaline Phosphatase Activity in Serum with Thymolphthalein Monophosphate. Clin Chem. 1970; 21:5.
25. Sood MM, Saeed M, Lim V, Cordova F, Komenda P, Malik A. 'The Urea-to-Creatinine Ratio is Predictive of Worsening Kidney Function in Ambulatory Heart Failure Patients'. J Card Fail. 2015; 21(5):412–418. https://doi.org/10.1016/j.cardfail.2015.02.003.
26. Lewis SM, Bain BJ, Bates I. Basic Haematological Techniques. In: Lewis, S.M., Bain, B.J. and Bates, I., Eds., Dacie and Lewis Practical Haematology, 10th Edition, Churchill Livingstone Elsevier, Philadelphia. 2006; 26-54. http://dx.doi.org/10.1016/B0-44-306660-4/50007-6.
27. Nwaka HC, Ezeabuchi SC. Effects of Aqueous and Methanol Extracts of *Newbouldia laevis* Leaves on Some Biochemical Makers of Wistar Albino Rats. Eur J Appl Sci. 2016; 8(5):270-276. DOI: 10.5829/idosi.ejas.2016.8.5.1141.
28. Kolawole OT, Adeyeba OA, Awodele O, Wakeel OK, Oluogun WA, Olaniyi OS. Evaluation of Chronic Toxicity of *Newbouldia laevis* Leaf Extract in Rats. Trop J Nat Prod Res. 2021; 5(10):1889-1895.
29. Nandy S, Datta R. 'Acute and Sub-acute Toxicity Studies of Methanol Leaves of *Pterospermium acerifolium* L. Willd in Rodents', Int J Pharm Life Sci. 2012; 3(3):1519–1529.
30. Kolawole OT, Akanji MA, Akiibinu MO. Toxicological Assessment of Ethanolic Extract of the Leaves of *Newbouldia laevis* (P. Beauv). Am J Med Med Sci. 2013; 3:74-80.
31. Joth SL, Zakaria Z, Chen Y, Lau YL, Ltha LY, Sasidharan S. 'Acute oral Toxicity of Methanolic Seed Extract of *Cassia fistula* in Mice'. Mol. 2009; 16(6):5268–5286. https://doi.org/10.3390/molecules16065268.
32. Waller DG, Sampson AP. Medical pharmacology and therapeutics. Elsevier, Edinburgh. 2018;5th edn.
33. Ping KY, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and Sub-chronic Toxicity Study of *Euphorbia horta* L. Methanol Extract in Rats. BioMed Res Int. 2013; 7:64-78. http://dx.doi.org/10.1155/2013/182064.
34. Chung GE, Yim JY, Kim D, Kwak M, Yang JI, Chung SJ, Yang SY, Kim JS. Association between White Blood Cell count and The Development of Incidental Nonalcoholic Fatty Liver Disease. Gastroenterol Res Pract. 2016; 7653689.
35. Iniagbe OM, Egharevba O, Oyewo EB. Effect of Aqueous Leaf Extract of *Acalypha wilkesiana* on Hematological Parameters in Male wistar albino rats. Br J Pharm Res. 2013; 3(3):465-47. doi: 10.1016/j.jep.2011.08.015.
36. Borén J, Chapman MJ, Krauss RM. Low-density Lipoproteins cause Atherosclerotic Cardiovascular Disease: Pathophysiological, Genetic, and Therapeutic Insights: A Consensus Statement from the European Atherosclerosis Society Consensus Panel. Euro Heart J. 2020; 41 (2020):2313-2330.
37. Akomas SC, Okafor AI, Ijioma SN. Hypolycemic, Hematologic and Hypolipidemic Activity of *Mucuna pruriens* Ethanol Leaf Extract in Alloxan Induced Diabetic Rats. Annu Res Rev Biol. 2014; 4(24):4284-4292.
38. Orieke D, Ohaeri OC, Ijeh II, Ijioma SN. Identification of Phytocomponents and Acute Toxicity Evaluation of *Corchorus olitorius* Leaf Extract. European J Med Plants. 2018. 23(1): 1-16.
39. Shrimanker I, Bhattarai S. *Electrolytes*. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2021; PMID: 31082167.
40. Ozer J, Ratner M, Shawc M. The Current State of Serum Biomarkers of Hepatotoxicity. Toxicol Letter. 2008; 245:194–205.
41. Hassan FI, Zezi AU, Danmalam UH, Yaro AH, Balarabe AN. 'Sub-chronic Toxicity Studies of the Methanol Leaf Extract of *Dalbergia saxatilis'*. Niger J Pharm Sci. 2015; 14(1):51–59.
42. Saptal SK, Punia JS. Studies on Biochemical Changes in Subacute Thiodicarb Toxicity in Rats. Toxicol. 2010; 17:30-32.
43. Iniagbe OM, Malomo SO, Adebayo JO, Arise RO. Evaluation of the Antioxidant and Hepatoprotective Properties of the Methanolic Extract of *Acalypha racemosa* leaf in Carbon tetrachloride-treated Rats. Afr J Biotechnol. 2008; 7(11):1716-1720