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Toxicological Assessment of Methanol Root Extract of *Newbouldia laevis* in Rats After Therapeutic Evaluation Against *Plasmodium berghei* in Mice

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ABSTRACT

This study evaluated the toxicity of methanol extracts of *Newbouldia laevis* in rats. Phytochemical screening was done using standard methods. Twenty five Wistar rats were divided into five groups of five rats each. The control group received 0.9% normal saline. The remaining groups were administered once daily for four days; 125, 250, 500, and 750 mg/kg body weight of the extract and afterwards monitored for 14 days. At the end, blood and organ samples were collected for biochemical, hematological and histopathological evaluation. Appreciable amount of flavonoids, alkaloids, saponins, tannins, oxalate and phytate were present. Significantly increased ALT activity of 65.28±1.55 U/L and 71.05±1.28 U/L were recorded at higher extract treatment doses of 500 and 750 mg/kg respectively compared to 34.71±1.99 U/L for the Control, just as AST and ALP presented similar trend of increased activities. Concentrations of total protein dropped from 74.74±2.25 g/dL (control) to 34.49±4.97 g/dL, while Urea (11.91±0.20 mg/dL) and creatinine (108.88±5.31 mg/dL) increased significantly compared to 5.36±0.41 mg/dL and 68.23±3.33 mg/dL recorded for controls respectively, indicating dose-dependent liver and kidney dysfunction. Other liver, kidney and haematological parameters varied significantly ($p \leq 0.05$) compared to the Control. Organ histology revealed pathological abnormalities in liver and kidney of treated rats at higher doses. This evaluation presented significant dose-dependent alterations in biochemical, haematological, histological parameters, indicating potential organ-specific toxicity at higher doses. These findings have shown *N. laevis* methanol root extract as a potential alternative treatment for malaria and a source for drug development having shown potent antiplasmodial properties, however, proper dose regulation is needed in the use.

Keywords: Medicinal plant, Phytochemicals, Biochemical, Haematological, Histological

Introduction

Malaria is a life-threatening disease transmitted through the bite of an infected Anopheles mosquito, causing significant morbidity and mortality globally.[1] Global malaria statistics show that as at 2023, an estimated 263 million cases of malaria were reported with 597,000 malaria deaths primarily (approximately 95%) occurring within Africa.[1] Malaria has a profound impact on global health, particularly in tropical and subtropical regions, where it is endemic.[2,3] Vulnerable populations affected by malaria include children less than five years, pregnant women, and people with compromised immune systems.[1] Efforts to control and eliminate malaria have been ongoing for decades, with a focus on vector control, diagnosis, and treatment.[1] However, malaria control is faced with significant challenge by the emergence of drug-resistant parasites and insecticide-resistant mosquitoes.[4] Therefore, continued research and development of effective and sustainable malaria control strategies are crucial to reducing the burden of this disease.

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Current treatments for malaria primarily involve the use of artemisinin-based combination therapies (ACTs) (WHO, 2020, however, the emergence of artemisinin-resistant parasites and the high cost of ACTs have resulted in a significant challenge to malaria control.[3,5,6] The development of new, effective, and affordable treatments for malaria is therefore critical to addressing these challenges and ultimately achieving the goal of malaria elimination. Plant-based remedies have shown potency and commonly used for ages in traditional medicine to treat various ailments, including malaria.[7,8,9] The importance of plant-based remedies lies in their potential to provide effective, affordable, and accessible treatments for malaria, particularly in resource-poor settings.[7] Many plant species have been found to possess antimalarial properties, and some have been shown to be as effective as conventional antimalarial drugs.[8,9] Plant-based remedies also offer a unique advantage in that they can be used in combination with conventional treatments to enhance their efficacy and reduce the risk of resistance.[11] The lack of effective monitoring and evaluation systems to track the efficacy and safety of herbal antimalarial treatments in Nigeria also poses a significant challenge.[12] Herbal treatments should have a favorable safety profile, minimizing the risk of adverse effects and improving treatment adherence. The search for new antimalarial treatments has led to increased interest in plant-based remedies, which have been used for centuries in traditional medicine.[7,13,14] *Newbouldia laevis* belongs to the Bignoniaceae family and it is a tropical tree plant that grows up to 3–8 m high. It grows in different regions of Nigeria especially in the western and southern regions and it is commonly called the following names in Nigeria: African hyssop, 'Ogirishi'' (by igbos of Eastern Nigeria), 'Àdùrúúkù'' (by Hausas of Northern Nigeria), and ''Akoko'' (by Yoruba of Western Nigeria).[15,16] *Newbouldia laevis* plant species have shown promising antimalarial activity, and this research was carried out to investigate the toxicological effects of methanol extracts. *N. laevis*, a plant species traditionally used in

Nigeria, was selected for this study due to its reported antimalarial properties.[15,16] The plant's widespread availability, affordability, and ease of preparation made it an attractive candidate for further investigation.

Malaria remains a significant global heath challenge, with the emergence of drug-resistant plasmodium strains necessitating the search for new and effective antimalarial agents which require thorough toxicological evaluation.[1,4] Toxicity test is a critical step in the development of new drug candidate to ensure safety for human consumption. Therefore, the aim of this study was to evaluate the toxicological profile of methanol root extract of *N. laevis* on Wistar rats with a view to establishing the extract safety as a potential antimalarial agent. Despite the importance of the use of plant extracts for malarial treatment due to increased resistance to synthetic drugs, the toxicological evaluation of such extracts is equally necessary to ensure the safety and tolerability

Materials and Methods

Materials

Chemicals/Reagents Used

All the chemicals used in this study were of analytical grade. BDH Methanol, Tween 80, Giemsa stain, phosphate buffer saline (PBS) of pH7.2, ethylene diamine tetraacetic acid (EDTA),0.1% ferric chloride, diluted ammonia, sulphuric acid, 20% acetic acid, ethanol, concentrated ammonium hydroxide, frothing reagent, olive oil, acetic anhydride, glacial acetic acid.

Plant Collection and Identification

Plant samples of *Newbouldia laevis* were collected in August 2015 from Umuezeala (Umudaranwaneri) village, located in Awo-Omamma, in Oru-East Local Government Area of Imo State, Nigeria. The coordinates were recorded using the Mobile Topographer application. The primary sampling point was georeferenced at latitude 5.66849354° N and longitude 6.95121404° E (WGS 84 datum), with an altitude of 158.60 meters above the ellipsoid. In Universal Transverse Mercator (UTM) coordinates (Zone 32N), this corresponded to Easting 273081.078 m and Northing 626960.199 m, with a height of 139.17 meters above mean sea level (MSL). The plant was identified by plant taxonomist Dr Francis Iwu of the Department of Forestry and Wildlife Technology at the Federal University of Technology Owerri (FUTO), Nigeria. The plant voucher number at "Ujor Forestry Harvester Ibadan is (FHI) 29271" and was deposited at the herbarium of the Department of Forestry and Wildlife Technology at the Federal University of Technology Owerri with a voucher number FUTO/H100125.

Plant Preparation and Extraction

The method of Buss and Butler[17] was adopted for the plant preparation and extraction. The plant roots were harvested in large quantities, washed thoroughly in tap water, cut into pieces, dried in the laboratory at room temperature for seven weeks and pulverized to powder using crusher machine. Using crude method, 100 g each of the pulverized parts were macerated in methanol, for 48 h and subsequently filtered using Whatman number 1 filter paper. The methanol filtrates were concentrated using water bath at 45^{O}C and the percentage yield determined. The extracts were stored in the refrigerator at 4^{O}C until required.

Phytochemical Screening

A portion of the extract was used for qualitative phytochemical screening. The phytochemical screening of methanol root extract of *N. laevis* plant was carried out to determine the secondary metabolites present therein using standard procedures. The method of Van-Burden and Robinson[18] was used to screen for tannin, Boham and Kocipai[19] for flavonoids, oxalate and phytate, Harborne[20] for alkaloids, phenols and steroids, Obadoni and Ochuko[21] for saponins, Trease and Evans[22] for terpenoids, and Sofowora[23] for cyanide glycoside.

Animals and Animal Husbandry

Twenty five Wistar rats used in this toxicological study were randomly divided into five groups of five rats and were treated as follows; Group 1, which was named Control group received Normal saline and the remaining groups (Groups 2-5) were administered graded doses of 125, 250, 500, and 750 mg/kg body weight (bw) of methanol root extract of *N. laevis* (Table 1). The extracts were administered once daily for four consecutive days. After the treatment period, all animals were monitored for another 14 days to observe any signs of toxicity or abnormal behavioural changes. Afterwards, the animals were fasted overnight and thereafter subjected to mild anesthesia, blood samples were collected by cardiac puncture and finally sacrificed to excise organ samples. The blood samples were used for biochemical and haematological analyses, and liver and kidneys samples were used for histopathological evaluation to assess possible organ-specific toxicity. The animals used in this study received professional humane care in compliance with the guidelines of Ethical Animal Handling and approval (FUTO/SOBS/BCH/2015/A023) was received from the ethics committee of the Department of Biochemistry, FUTO, Nigeria.

Determination of liver function parameters

Liver panel was determined by using Randox assay kits by assaying the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by the method described by Reitman and Frankel.[24]

The alkaline phosphatase (ALP) activities were assayed by the optimized standard method described by Roy.[25] Total protein was determined colorimetrically as described by Tietz[26] using Randox assay kits. Concentration of albumin was determined according to Doumas et al.[27] method, urea and creatinine concentrations were determined as described by Chaney and Marbach.[28] Serum sodium and potassium were determined according to the method of Tietz[29], and Henry[30], respectively. The concentration of serum bicarbonate was determined as described by Tietz.[31]

Determination of Hematological Parameters

The determination of haematological parameters was by the method of Dacie and Lewis.[32] Sysmex KX-21N automatic multi-parameter blood cell counter was adopted.[33] Blood cell count was carried out by the volumetric impedance method. This method directly counts blood cells by detecting changes in electrical resistance as cells pass through an aperture in Sysmex KX-21N.[34] As the movement was maintained it directly measured WBCs, RBCs, hemoglobin, platelets, MCV, and mean platelet volume. From these values, additional parameters like hematocrit, MCH, MCHC, and distribution widths were automatically calculated to evaluate the full blood composition

Histological Studies of Heart, Liver and Kidney Tissues

To ascertain liver and kidney organ health, histological examination was conducted on the samples obtained from the treatment groups of animals, adopting the method of Okoro[35] with slight modifications. The tissues were fixed in a large volume of 10% formaldehyde, with complete coverage of the organs, before undergoing further processing. The organs were dehydrated by passing them through increasing concentrations of alcohol (30%, 50%, 70%, 90%, and absolute alcohol) for 1h, 2h, and 3h each. Following dehydration, the tissues were immersed in xylene for 3 h to remove residual alcohol, then transferred to a bath containing molten paraffin wax in an embedding oven. During embedding, the clearing agent diffused out of the tissues into the wax. The Organs were subjected to other standard processes such as embedding, trimming, sectioning, attaching sections to sides, and staining. The staining process began with dewaxing and hydrating the section. To achieve this, the section was first subjected to 100°C on a hot plate for a brief period, and the wax was dissolved by immersing in xylene for 30 min. Afterwards residual xylene was removed by immersing in absolute alcohol for 30s. The section was passed through a series of decreasing alcohol concentrations, starting with 90% alcohol for 30 s, followed by 70% alcohol for another 30 s. After alcohol treatment, the section was washed thoroughly in distilled water to remove any remaining alcohol. The section was then treated with a differentiating solution until only the cell nuclei retained the stain. To restore the natural colour of the tissue, the section was blued using Scott's tap water substitute under running tap water for 5 min. Finally, the section was counterstained with eosin for 1 min to enhance the visibility of the tissue structures. The section was dehydrated by using

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ascending grades of alcohol, cleared in xylene, and mounted using dibutylphthalate, polystyrene, xylene (DPX) as the mounting medium and was examined using a light microscope and photographed at a magnification of x400.

Statistical Analysis

All data obtained from the study were subjected to analysis of variance (ANOVA) using the SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). Results were presented as mean ± standard deviation (SD). Post hoc multiple comparison tests were carried out to evaluate differences between group means, with statistical significance set at $p \leq 0.05$.

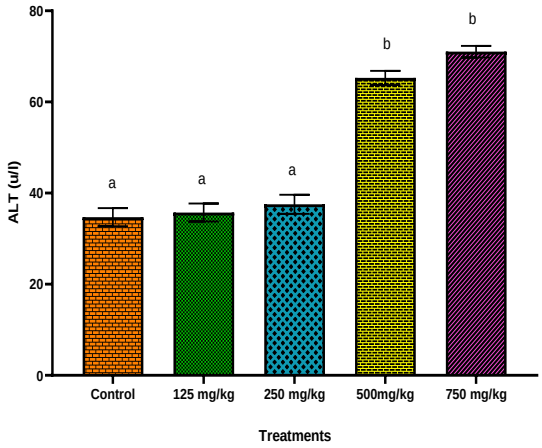
Results and Discussion

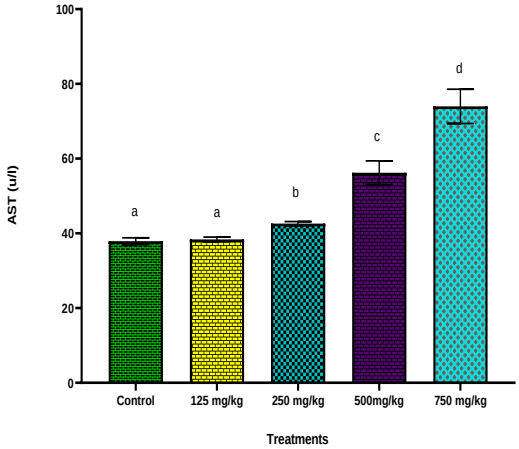
This study evaluated the toxicological profile of methanol root extract of *N. laevis* after the administration to Wistar rats with a view to establishing the safety of methanol root extract of *N. laevis* as a potential antimalarial agent. It is important to state here that just as the antimalarial efficacy of plant based treatments remains a critical area of scientific study due to increased resistance to synthetic drugs, the toxicological evaluation of such extracts is equally necessary to ensure the clinical safety and tolerability.[33,36] Studies have shown that numerous plant extracts present potential pharmacological activity but are sometimes overlooked as a result of inadequate safety data.[37] In this study the liver function parameters shown in Figure 1 presents liver enzyme activities and protein concentrations of rats treated with *N. laevis* extract. ALT activity significantly increased in rats treated with 750 mg/kg (71.05±1.28 U/L) and 500 mg/kg extracts compared to Control group (34.71±1.99 U/L). AST rose to 73.99±4.59 U/L (750 mg/kg) from 37.89±0.93 U/L (control), while ALP increased to 126.77±3.83 U/L (750 mg/kg) compared to 67.74±11.87 U/L (Control). At low dose administration of 125 and 250 mg/kg *N. laevis* extract-treated rats had serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities that were comparable to the control group, indicating absence of hepatocellular injury or cholestasis. However, administration of higher (750 mg/kgbw) methanol root extract of *N. laevis* showed significant elevation of activities of ALT (71.05±1.28 U/L), AST (73.99±4.59 U/L), and ALP (126.77±3.83 U/L) compared to the control group. This elevated serum ALT and AST activities on test rats compared to control are attributable to damages on the structural integrity of the liver[38], leading to liver membrane dysfunction and cellular leakage enzymes.[39]

Table 1: Experimental Design

Groups	Group Identity	Treatment
Group 1	Control	Normal saline
Group 2	Low Dose	125 mg/kg of Methanol Root Extract
Group 3	Medium Dose	250 mg/kg of Methanol Root Extract
Group 4	High Dose	500 mg/kg of Methanol Root Extract
Group 5	Very High Dose	750 mg/kg of Methanol Root Extract

The recorded elevation observed in this study indicates that higher doses of *N. laevis* extract may induce hepatocellular damage. Similar hepatotoxic effects have been reported in studies involving high doses of *N. laevis* extracts.[16,40] These variations can be attributed to the presence of phytochemicals in methanol root extract of *N. laevis* such as alkaloids, tannins, and saponins. These phytochemicals are implicated to directly damage hepatocyte membranes causing leakage of cytosolic enzymes into the bloodstream.[41] Figure 1 also showed that total protein dropped from 74.74±2.25 g/dL (control) to 34.49±4.97 g/dL, and albumin from 43.18±3.73 g/dL to 19.39±0.69 g/dL. A dose-dependent decrease in total protein and albumin concentrations was recorded, with the 750 mg/kg group showing the lowest concentrations (total protein: 34.49±4.97 g/dL; albumin: 19.39±0.69 g/dL). The significant dose-dependent decrease in total protein and albumin concentrations may be attributed to impaired hepatic synthetic function or increased protein loss, which is in line with report of Aderinola et al.[16], and Murtala et al.[40] on *N. laevis* toxicity. Albumin, in particular, is produced exclusively by hepatocytes and decreases significantly only when the synthetic capacity of the liver is compromised over a longer period.[42,43] The recorded reduction in albumin concentration clearly indicates that higher doses of methanol root extract of *N. laevis* may disrupt hepatic protein production. Phytochemicals such as pyrrolizidine alkaloids or anthraquinones have been implicated to impairing albumin synthesis executed by damaging hepatocytes, disrupting ribosomal function, or triggering inflammatory responses. This effect is dependent on the type, dose, and combination of phytochemicals.[37,44,45,46]





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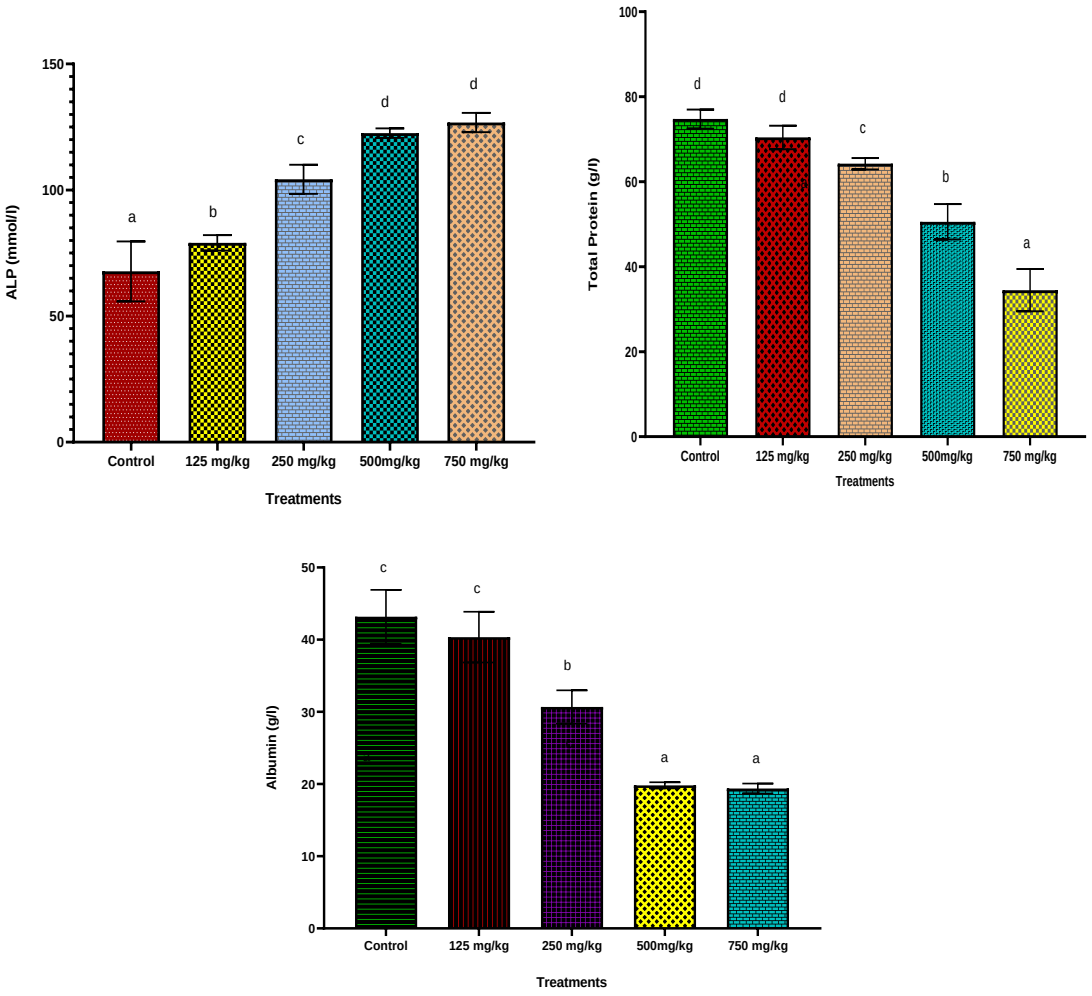

Figure 1: Liver enzyme activities and protein concentrations of rats treated with different doses of methanol root extract of *N. laevis*. Values are presented as Mean ± Standard deviation of quadruple determination. Bars bearing different superscripts indicate significant difference at $p < 0.05$

The results (Figure 2) of kidney functions showed a dose-dependent rise in urea and creatinine concentrations. Urea significantly increased to 11.91±0.20 mg/dL (750 mg/kg) compared to 5.36±0.41 mg/dL (control), and creatinine rose to 108.88±5.31 mg/dL compared to the control at 68.23±3.33 mg/dL. The significant increases in urea and creatinine concentrations were recorded at higher *N. laevis* extract doses, with the 750 mg/kg group showing the highest concentrations of urea and creatinine. Elevated concentrations of urea and creatinine are biomarkers indicative of compromised renal function.[47] Furthermore, the observed dose-related increases in serum urea and creatinine concentrations accompanied by progressive hyponatraemia and hypokalaemia, and non-significant mild rise in bicarbonate concentration at the highest dose—points to combined glomerular and tubular injury. Similar outcome has been reported for ethanol leaf extract of *Hypoestes rosea*, in which the histology of tissues confirmed dose-dependent glomerular shrinkage and tubular necrosis.[48] Electrolyte concentration results (Figure 2) showed that sodium decreased with the dose dropping to 138.23±3.98 mmol/L (750 mg/kg) compared to 143.09±2.60 mmol/L in Control. Potassium also declined to 4.11±0.03 mmol/L (750 mg/kg). The decrease in serum concentrations of Na^+ and K^+, may be attributed to disturbance in renal tubular ion handling.[49] Furthermore, Bicarbonate concentrations slightly increased, with 750 mg/kg group showing 26.04±3.31 mmol/L compared to 24.41±1.54 mmol/L (control). This modest bicarbonate rise may indicate a mild metabolic compensation typical of early renal dysfunction.[49] The presence of appreciable amount of alkaloids, tannins, saponins flavonoids etc. (Table 2), recorded in methanol root extract of *N. laevis* can be attributed to the biochemical effects recorded.

Table 2: Qualitative screening of methanol root extract of *N. laevis*.

Phytochemical	Results
Tannins	+
Saponins	+
Flavonoids	+
Cyanide Glycoside	+
Terpenoids	-
Steroids	-
Alkaloids	+
Phenol	+
Oxalate	+
Phytate	+

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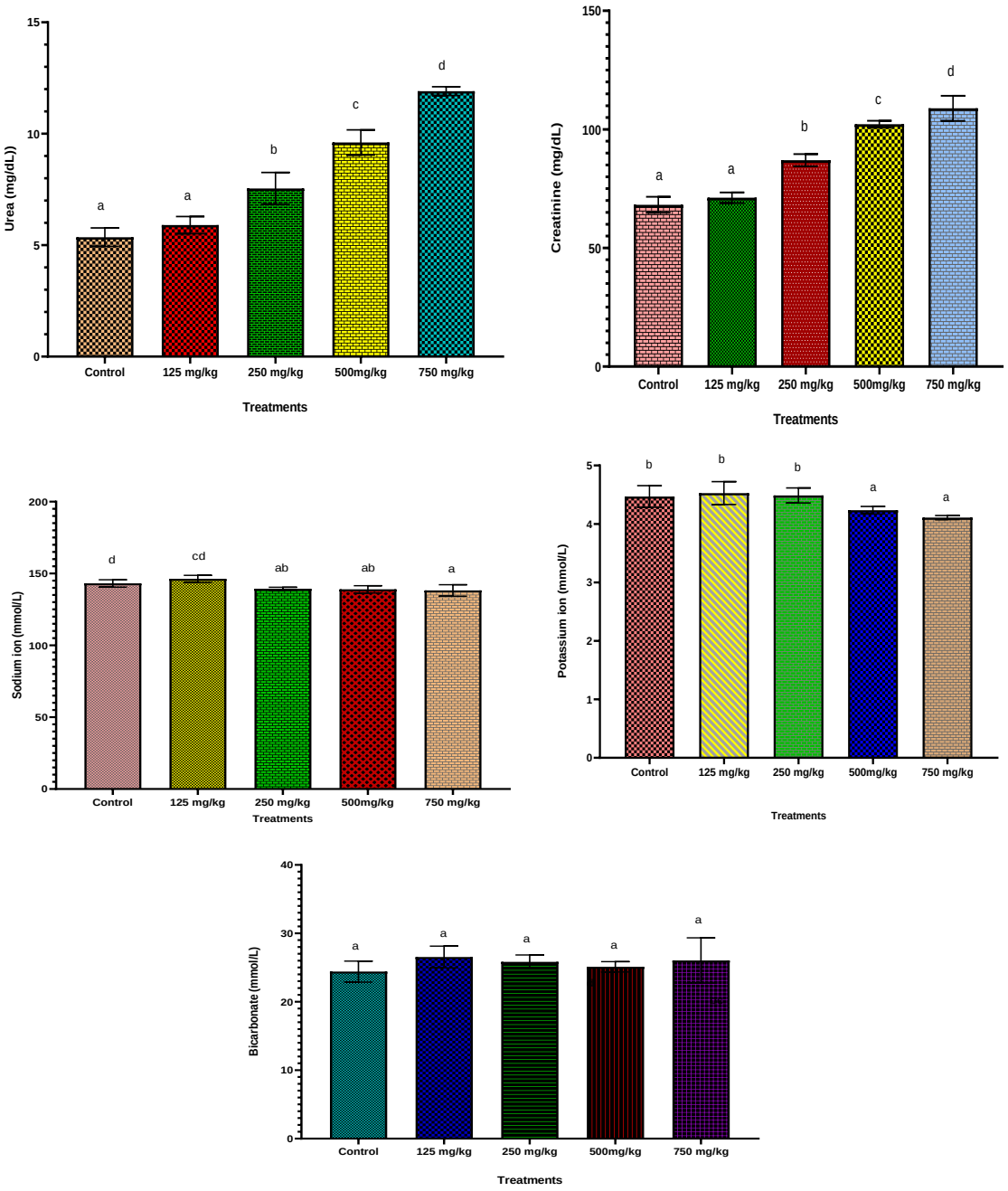

Figure 2: Kidney function profile of rats treated with different doses of methanol root extract of *N. laevis*. Values are presented as Mean ± Standard deviation of quadruple determination. Bars bearing different superscripts indicate significant difference at $p < 0.05$.

This aligns with the work of Eneh et al.[50] who reported appreciable levels of alkaloids, saponins, tannins, flavonoids, terpenes and anthraquinones in *N. laevis* leaves. Of the phytochemicals determined, alkaloids are indicated as the prime nephrotoxic candidate. Studies have shown that some alkaloids are bio-activated in the kidney to reactive species that trigger oxidative stress, mitochondrial damage and inflammatory cascades, impair filtration and electrolyte reabsorption.[51] Saponins and tannins can further destabilise tubular membranes, exacerbating ion loss, while high-dose anthraquinones have been shown to upset bile-acid and renal transport processes amplifying functional decline of the kidney. Therefore, the observed dose-dependent biochemical derangements observed in this study can be attributed to the cumulative toxic actions of these phytochemical classes acting on both glomerular and tubular segments of the nephron. The haematological results (Table 3) showed significant decreases in haemoglobin, RBC, WBC, and PCV concentrations at 500 mg/kgbw and 750 mg/kgbw doses. The acute toxicity study of *N. laevis* methanol root extract reveals significant dose-dependent haematological alterations in rats, which can be attributed to the phytochemical contents of the plant. These phytochemicals can interact with biological systems and affect haematological parameters.

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Table 3: Haematological contents of rats treated with different doses of methanol root extract of *N. laevis*.

Groups	Hb (g/dL)	RBC (×10⁶/µL)	WBC (×10³/µL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PCV (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Control	15.55±0.57^{c}	2.48±0.24^{d}	8.45±0.66^{c}	181.07±22.21^{ab}	63.22±5.98^{a}	35.01±1.36^{ab}	44.50±3.11^{bc}	53.75±2.87^{a}	46.75±3.40^{b}	1.50±0.58^{a}	0.25±0.50^{a}	0.00±0.00^{a}
125 mg/kg	14.80±1.14^{c}	2.15±0.19^{c}	8.58±0.48^{c}	213.75±16.01^{bc}	69.18±7.37^{a}	32.41±3.13^{c}	45.75±1.71^{c}	53.50±3.42^{a}	44.75±3.40^{b}	1.75±0.50^{a}	0.25±0.50^{a}	0.00±0.00^{a}
250 mg/kg	14.85±0.75^{c}	2.18±0.10^{c}	7.95±0.34^{c}	192.23±15.13^{bc}	68.40±5.02^{a}	35.60±0.72^{bc}	41.75±2.75^{bc}	52.00±2.83^{a}	44.00±3.65^{b}	1.75±0.96^{a}	0.00±0.00^{a}	0.00±0.00^{a}
500 mg/kg	11.90±1.41^{b}	1.79±0.21^{b}	5.73±0.68^{b}	223.02±21.30^{c}	66.82±5.28^{a}	30.16±3.80^{a}	39.50±1.91^{b}	52.00±2.83^{a}	47.25±2.22^{b}	3.00±0.82^{b}	0.00±0.00^{a}	0.00±0.00^{a}
750 mg/kg	9.10±1.16^{a}	1.46±0.10^{a}	4.18±0.46^{a}	153.49±31.17^{a}	62.42±6.75^{a}	41.99±9.46^{d}	22.50±5.51^{a}	53.75±3.86^{a}	38.75±2.75^{a}	2.75±0.96^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values are represented as Mean ± Standard deviation of quadruple determination. Bars bearing different superscripts indicate significant difference at $p < 0.05$.

Mean corpuscular volume (MCV) increased in rat groups administered 500 mg/kg of extract but dropped in 750 mg/kg extract group, indicating potential macrocytosis, while the decreased MCV at 750 mg/kg which indicates microcytosis, also reflected dose-dependent effects on red blood cell morphology.[52,53] Mean corpuscular haemoglobin concentration (MCHC) peaked at 750 mg/kg group with a concentration of 41.99±9.46 g/dL. Elevated MCHC at 750 mg/kg may indicate hyperchromic cells, while the decreased MCHC at 500 mg/kg suggests hypochromic cells. These variations in MCV and MCHC could be indicative of disrupted erythropoiesis or haemolysis at higher extract concentrations.[40] Higher doses (500 and 750 mg/kg) of the *N. laevis* extract induced a marked dose-dependent decline in Hb, RBC, WBC, and PCV, indicating anemia and potential immunosuppression. The observed significant reduction in WBC count at higher doses implies potential immunosuppressive effects of the extract.[54] Furthermore, Table 2 showed that lymphocyte counts varied, with the lowest (38.75±2.75 %) recorded in 750 mg/kg extract group. Monocyte levels rose significantly at higher doses with 3.00±0.82% and 2.75±0.96% at 500 mg/kg and 750 mg/kg respectively. However, the lack of significant changes in neutrophil and eosinophil counts implies that specific leukocyte populations may have been differentially affected.[53] Furthermore, the increase recorded in monocyte percentage at higher doses of 500 mg/kg and 750 mg/kg could be attributed to an inflammatory response or compensatory mechanism, potentially mediated by the saponins and alkaloids.[40] Alkaloids (Anthraquinones) are reported to generate reactive oxygen species which depresses marrow erythropoiesis and damage RBC membranes, driving the anaemia and macrocytic shift.[55,56]

Saponin creates pores in cholesterol-rich erythrocyte membranes, promoting haemolysis and compensatory macrocytosis[57], while tannins are binders of membrane proteins, potentiating oxidative fragility and further lowering RBC survival[58]. These findings align with studies on other methanol extracts of other plants. For instance, a study on *Syzygium guineense* methanol leaf extract showed no adverse haematological effects at doses up to 5000 mg/kg, which indicated species-specific responses to methanol extracts.[54] Conversely, methanol leaf extract of *Alchornea cordifolia* presented significant decreases in erythrocyte count, Hb concentration, and PCV at 1600 mg/kg, and was attributed to haematotoxicity at high doses.[16] The histological analysis of liver sections (Figure 3) from rats administered methanol root extract of *N. laevis* revealed dose-dependent hepatic alterations, indicating potential toxicity. Control liver sections (LV1) showed normal architecture, intact hepatocytes, and organized sinusoids, reflecting proper liver function.[59] At 125 mg/kg (LV.2), mild hepatocellular depletion and nuclear degeneration were evident, suggesting oxidative stress from phytochemicals such as alkaloids.[60,61] At 250 mg/kg (LV3), sclerotic vessels and enlarged hepatocytes with abnormal nuclei suggested impaired perfusion and inflammation, typical of toxic injury.[62,63] Hepatocyte enlargement (ballooning degeneration) is a classic feature of toxic hepatic injury.[64] The 500 mg/kg dose (LV4) revealed arteriosclerotic vessels and lipid accumulation, indicating disrupted lipid metabolism and oxidative stress.[65,66]

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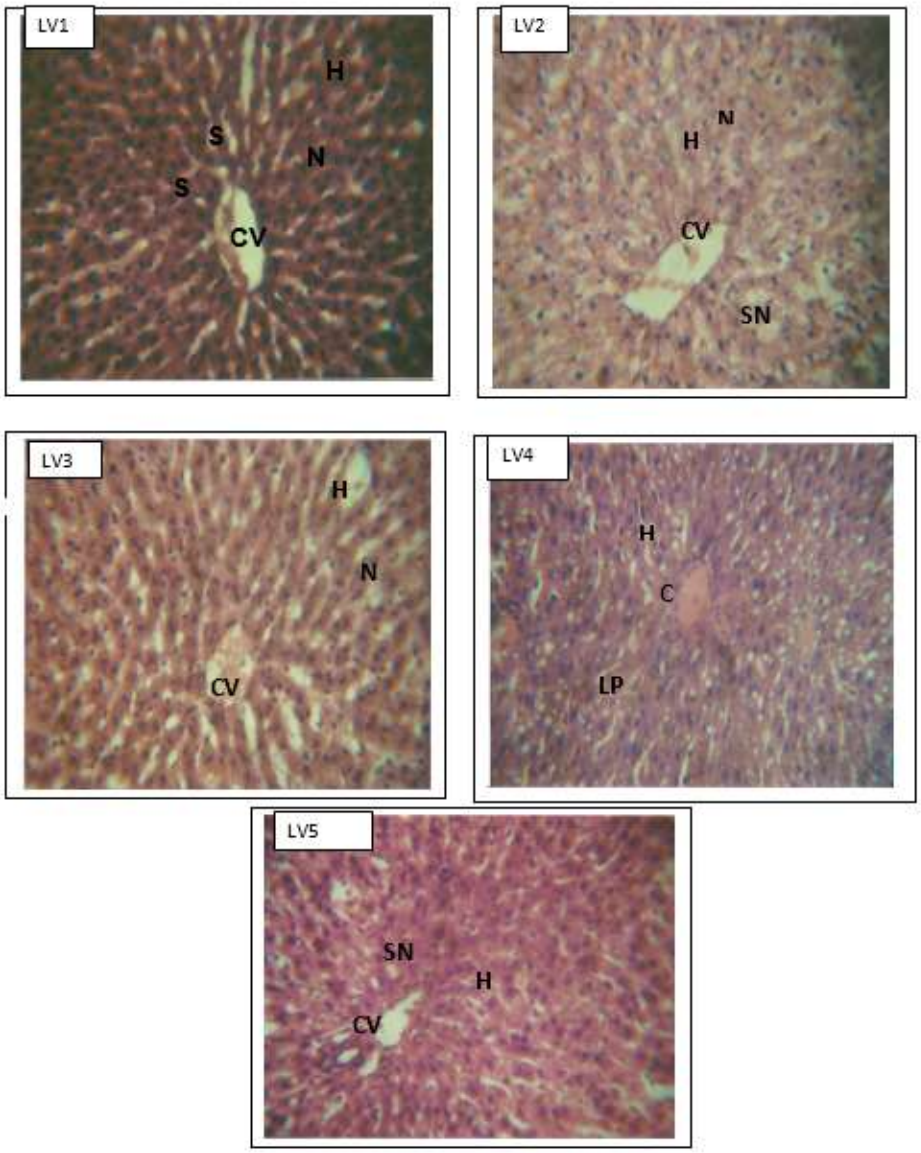

Figure 3: Histology of liver of rats following sub-acute administration of 125, 250, 500 and 750mg/kgbw of methanol root extract of *N. laevis* (x400). LV1 Control, LV2- administered 125 mg/kg, LV3- administered 250 mg/kg, LV4- administered 500 mg/kg, LV5- administered 750 mg/kg. KEY: CV= central vessel, SN= Sinusoid, H = Hepatocytes, N = Nuclei, LP = Lipids

Severe degenerative changes were observed at dose 750 mg/kgbw (LV5), including vessel constriction and disorganized hepatic cords, signaled significant hepatocellular damage and possible early fibrosis.[67,68] These findings indicate that while *N. laevis* contains therapeutic phytochemicals, higher doses may exert hepatotoxic effects through oxidative stress and mitochondrial disruption.[69,70] Similarly, the histological assessment of the kidney sections following sub-acute administration of *N. laevis* methanol root extract indicated dose-dependent renal architectural changes (Figure 4). The Control group rats (KD1) showed normal kidney cytoarchitecture (indicated by intact glomeruli, Bowman's capsules, and renal tubules) indicating optimal filtration and reabsorption functions.[59] At doses 125 and 250 mg/kgbw (KD2 and KD3), renal tissues remained largely intact, aligning with evidence that certain phytochemicals may offer nephroprotective benefits at therapeutic levels.[69] Rats administered 500 mg/kg (KD4), presented slightly widened urinary space, an early indicator of glomerular stress or vascular disruption, possibly due to mild oxidative stress.[67] At 750 mg/kgbw (KD5), extensive degeneration of glomerular, tubular, and connective tissues was observed, indicating impaired renal filtration and possible acute kidney injury. These adverse structural changes may be linked to high concentrations of phytochemicals such as saponins and alkaloids, which are cytotoxic at high doses.[66,70,71] These results imply that, while *N. laevis* may be safe at low doses, its nephrotoxic potential at higher concentrations underscores the importance of dose regulation in therapeutic applications.

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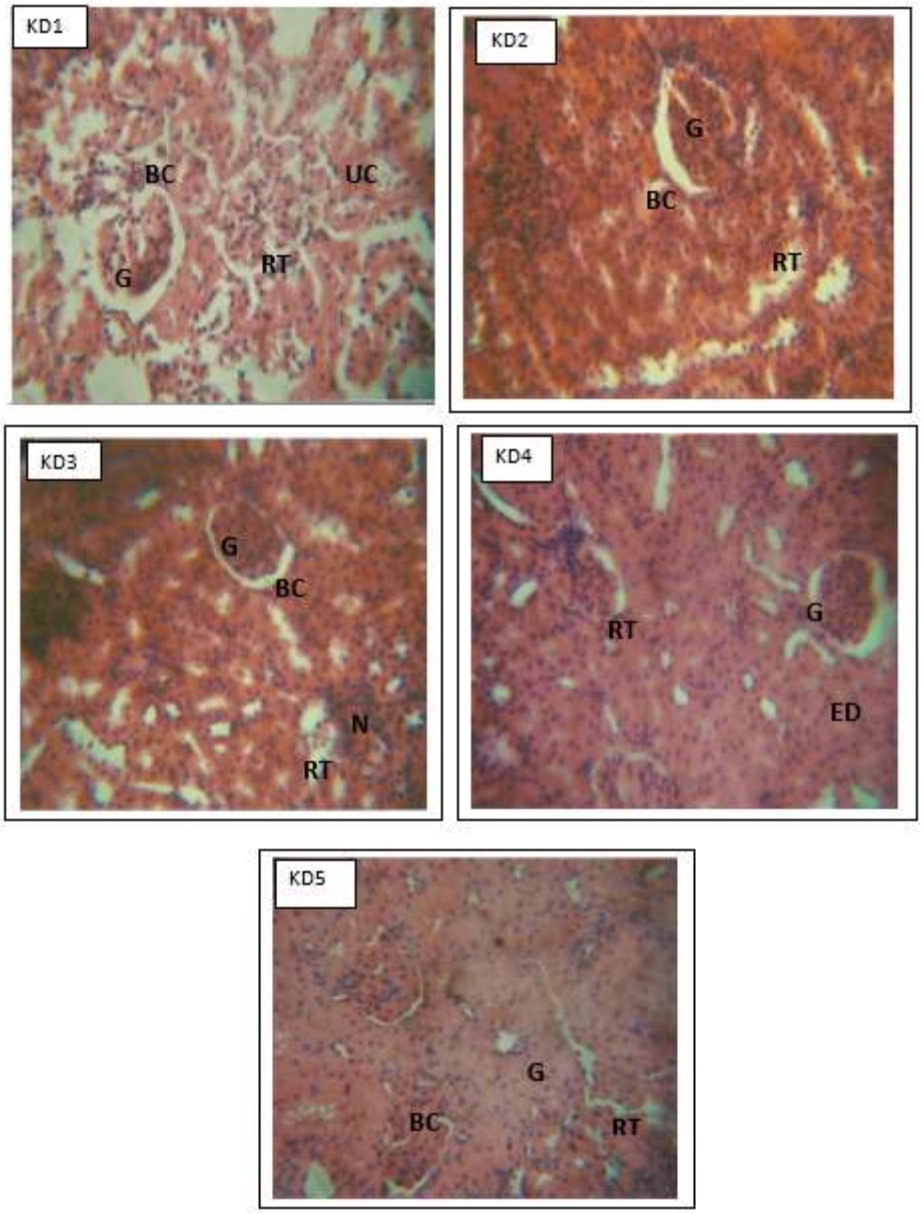

Figure 4: Histology of kidney of rats following sub-acute administration of 125, 250, 500 and 750mg/kg of methanol root extract of *N. laevis* (x400). KD1= Control, KD2- administered 125 mg/kg, KD3- administered 250 mg/kg, KD4- administered 500 mg/kg, KD5- administered 750 mg/kg. KEY: G= glomeruli, RT = renal tubules, UC - urinary capsule, N= nuclei, ED= endothelium of the glomeruli

Conclusion

The findings from this study indicated that acute administration of *N. laevis* methanol root extract at higher doses can lead to significant hepatic and renal toxicity, as evidenced by elevated liver enzymes, urea, and creatinine levels. Additionally, disturbances in protein synthesis, electrolyte balance, and lipid profiles were observed. These results underscore the importance of cautious dosing when considering *N. laevis* for medicinal use and highlight the need for further research to establish safe therapeutic ranges.

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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