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Acute Oral Toxicity of an Alkaloid-Rich Fraction of *Nauclea diderrichii* Stem Bark in Wistar Rats: Biochemical and Histopathological Evaluation

[1]Ibrahim Hamisu Abdul, *[1]Alhassan Muhammad Wudil, [2]Abubakar Yusuf

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University Kano, Kano State, Nigeria.
[2]Department of Biochemistry, Faculty of Science, Federal University, Gashua, Yobe State, Nigeria.

*Correspondence: amwudil.bch@buk.edu.ng

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ABSTRACT
The study evaluated the acute oral toxicity of an alkaloid-rich fraction derived from the stem bark of *Nauclea diderrichii*, a plant traditionally used in Sub-Saharan Africa for various ailments including malaria and gastrointestinal disorders. Acute oral toxicity was assessed in Wistar rats using Lorke's method (Phase I: n = 3 per group; Phase II: n = 1 per dose). A separate cohort of male Wistar rats (n = 3 per group: vehicle control, 1000, 3000 and 5000 mg/kg) was used to evaluate body weight, relative organ weight, serum biochemical biomarkers and histopathology. No mortality was recorded at any dose up to 5000 mg/kg, indicating an oral LD_{50} greater than 5000 mg/kg. Minor, transient behavioural changes such as lethargy and reduced feeding were observed at higher doses and resolved within 24 h. Body-weight gain was modestly reduced, and relative liver and kidney weights were significantly elevated at 3000 and 5000 mg/kg. Serum ALT, AST, ALP, bilirubin, urea, creatinine and cystatin C increased in a dose-dependent manner at these doses, while histopathological evaluation revealed no structural lesions in liver or kidney at any dose. The absence of mortality up to 5000 mg/kg suggests low acute lethality; however, the dose-dependent biochemical and organ-weight changes observed at higher doses indicate biological effects that warrant further investigation. The findings of this study served as the foundation of safety guide of traditional preparations of *N. diderrichii* and further sub-acute/chronic studies incorporating sensitive structural biomarkers are needed before therapeutic or ethnomedicinal safety conclusions can be drawn.

Keywords: *Nauclea diderrichii*, Alkaloid-Rich Fraction, Acute Oral Toxicity, Biomarker, Histopathology, Hepatorenal

INTRODUCTION

Medicinal plants remain indispensable sources of primary healthcare delivery and in the discovery pipeline for new therapeutic agents, particularly across sub-Saharan Africa, where herbal remedies remain the first point of care for a large proportion of the population (Moyo *et al*., 2015; Latif and Nawaz, 2026). Alkaloids constitute one of the most pharmacologically versatile classes of plant secondary metabolites, with documented antimicrobial, antimalarial, anti-inflammatory, analgesic, and antioxidant activities (Yang *et al*., 2024). Despite these biological activities, numerous alkaloids have narrow therapeutic windows and may cause adverse effects on the liver, kidneys, nervous system, and cardiovascular tissues when consumed at inappropriate doses or following prolonged exposure (Lamidi *et al*., 2005; Thakur *et al*., 2024; Shan *et al*., 2026). Establishing the acute toxicological profile of alkaloid-enriched plant fractions is therefore a prerequisite for their rational and evidence-based therapeutic application.

The liver and kidneys are particularly susceptible to phytochemical-induced injury because they serve as the principal organs responsible for xenobiotic metabolism, biotransformation, and excretion (Pearson *et al*., 2022; Qadri *et al*., 2025). Following oral administration, plant-derived alkaloids undergo extensive hepatic metabolism, generating metabolites that may either enhance therapeutic efficacy or elicit toxic responses through oxidative stress, mitochondrial dysfunction, disruption of membrane integrity, inflammatory signalling, and apoptotic pathways (Gu and Manautou, 2012; Dey *et al*., 2020; Messina and Duclos-Vallée, 2023). Likewise, renal elimination exposes nephron structures to circulating xenobiotics and their metabolites, making renal tissue especially vulnerable to functional impairment (Pearson *et al*., 2022). Although these mechanisms have been described for several alkaloids, the toxicological behaviour of many ethnomedicinal plant species remains insufficiently investigated, particularly those widely used in African traditional medicine (Moyo *et al*., 2015).

Nauclea diderrichii (De Wild. & T. Durand) Merr., a member of the *Rubiaceae* family, is widely distributed across tropical West and Central Africa and occupies an important position in indigenous healthcare systems. Various parts of the plant have traditionally been employed for the treatment of malaria, fever, gastrointestinal disorders, microbial infections, and inflammatory diseases. Phytochemical investigations have identified numerous bioactive constituents, including indole alkaloids, quinovic acid glycosides, flavonoids, tannins, and phenolic compounds, many of which have demonstrated antimicrobial, antiplasmodial, antioxidant, and anti-inflammatory activities (Akunne *et al.*, 2017; Abdulrahman and Adamu, 2019; Douho Djimeli *et al.*, 2022). These findings support the growing pharmacological interest in *N. diderrichii* as a potential source of novel therapeutic agents while simultaneously emphasizing the need for systematic safety evaluation of its bioactive fractions (Mbiantcha *et al.*, 2020; Kuete and Seukep, 2023).

Acute toxicity, cytotoxicity, organ dysfunction and structural organ injury are related but distinct concepts that must not be used interchangeably. Acute toxicity describes adverse effects, including mortality, occurring within a short period

after a single or short-term exposure; cytotoxicity refers to direct injury to cells in culture or simple organisms and does not by itself establish whole-organism toxicity; organ dysfunction denotes an alteration in a functional index (e.g., a serum enzyme or metabolite) that may or may not reflect irreversible tissue damage; and structural organ injury refers specifically to histologically demonstrable damage to tissue architecture. Acute toxicity studies provide the first experimental evidence regarding the immediate biological effects of a substance following a single or short-term exposure. Such investigations establish the median lethal dose (LD_{50}), identify early clinical manifestations of toxicity, evaluate target-organ susceptibility, and generate baseline information required for subsequent sub-chronic and chronic toxicity studies (Akhila *et al.*, 2007). The integration of biochemical biomarkers with histopathological examination substantially improves the reliability of acute safety assessment by combining functional indices of organ integrity with direct microscopic evaluation of tissue architecture (Kilty *et al.*, 2007), since these two categories of endpoint are not interchangeable and may legitimately diverge.

Despite the increase of its pharmacological significance, systematic toxicological evaluation of the alkaloid-rich fraction of *N. diderrichii* stem bark remains limited. Existing studies have predominantly emphasized phytochemical composition and pharmacological activities, whereas systematic evaluation of its acute oral safety using integrated biochemical and histopathological approaches has received little or no attention, leaving open the possibility that dose-dependent biomarker changes are misinterpreted as evidence of overt organ damage when no corresponding structural lesion exists. This represents a gap in the safety literature, given that such misclassification can restrict the therapeutic development of an otherwise pharmacologically promising plant fraction.

We hypothesised that high-dose administration of the alkaloid-rich fraction would produce measurable biochemical evidence of hepatic and renal perturbation without a corresponding, histologically detectable structural lesion, reflecting a dissociation between functional and structural injury markers rather than overt organ damage. Accordingly, the present study investigated the acute oral toxicity of an alkaloid-rich fraction obtained from the stem bark of *Nauclea diderrichii* using Wistar rats, combining clinical observations, body and organ weight assessment, biochemical evaluation of hepatic and renal function, and histopathological examination to generate baseline toxicological data that will contribute to the safe medicinal use and future pharmacological development of this important African medicinal plant.

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh stem bark of *Nauclea diderrichii* was collected, and the plant material was identified and authenticated by a taxonomist in the Department of Biology, Bayero University, Kano, Nigeria. A voucher specimen (Voucher No: BUKHAN 0434) was deposited in the departmental herbarium for future reference. The stem bark samples were washed with distilled water and air-dried under shade at room temperature (25–28°C) for two weeks to prevent degradation of thermolabile phytochemicals.

Chemicals and Reagents

Analytical grade chemicals and reagents were used throughout the study, including methanol, chloroform, hydrochloric acid, ammonia solution, Dragendorff's reagent, Wagner's reagent, sodium chloride, formalin, haematoxylin and eosin stain. Commercial diagnostic kits used for biochemical analysis of liver and kidney function biomarkers are detailed under Biochemical Analysis below.

Ethical Approval

All experimental procedures involving animals were approved by the Animal Care and Use Research Ethics Committee, Bayero University, Kano, Nigeria (Approval Number: BUK/ACUREC/CAP/PG223), and were conducted in accordance with the guidelines of the National Research Council (2011) for the care and use of laboratory animals and the OECD principles for laboratory animal use.

Experimental Animals

Healthy adult male Wistar rats weighing between 180 and 200 g were obtained from the Animal House Facility, Department of Human Physiology, Bayero University, Kano, Nigeria. Only male animals were used to avoid the confounding influence of the oestrous cycle on body-weight, organ-weight and biochemical endpoints. The animals were housed in clean polypropylene cages under standard laboratory conditions of temperature (25 ± 2°C), relative humidity (55–65%), and a 12 h light/dark cycle, with standard laboratory feed and water provided ad libitum. Animals were allocated to groups by simple randomisation, and cage allocation was rotated to minimise positional bias.

Overview of Experimental Design

Two related but distinct components make up the acute toxicological evaluation reported here. The first is the classical Lorke procedure, used exclusively to establish clinical signs of toxicity, mortality, and the median lethal dose (LD_{50}); this comprised Phase I (10, 100 and 1000 mg/kg, n = 3 rats per dose) and Phase II (1500, 3000 and 5000 mg/kg, n = 1 rat per dose, used for dose-range finding as prescribed by the Lorke method). Because Phase II used a single animal per dose, no variance statistics were computed for this component, and the corresponding results are reported descriptively (Table 3). The second component is an independently constituted toxicological cohort, comprising a vehicle-treated control group and three treatment groups (1000, 3000 and 5000 mg/kg), each with n = 3 male rats, run concurrently with the Lorke procedure. This cohort was used to generate the body-weight, relative organ-weight, serum biochemical and histopathological data reported in Tables 4–6 and Figures 1–2. These two reporting components are apparent between the Lorke dose-finding design and the quantitative statistical analyses.

Extraction and Preparation of the Alkaloid-Rich Fraction

Powdered stem bark (600 g) was macerated in methanol (1:5 w/v, 2.5 L) for 72 h with intermittent agitation (Harborne, 1998). The macerate was filtered (Whatman No. 1) and concentrated under reduced pressure at 40 °C using a rotary evaporator to yield the crude methanolic extract. The alkaloid-rich fraction was subsequently isolated by acid-base solvent partitioning (Evans, 2009): the crude extract was acidified with 2% hydrochloric acid, filtered to remove insoluble matter, and the filtrate basified to pH 9–10 with concentrated ammonia solution before repeated liquid–liquid extraction with chloroform. Pooled chloroform fractions were concentrated under reduced pressure to yield a dark-brown alkaloid-rich fraction, which was stored at 4 °C until use. Percentage yield was calculated as:

% Yield = (Weight of extract / Weight of plant material) × 100

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the alkaloid-rich fraction was performed using standard protocols to test for alkaloids, flavonoids, saponins, and terpenoids (Evans, 2009; Shaikh and Patil, 2020). The presence of alkaloids was specifically confirmed by the formation of characteristic precipitates with Dragendorff's and Wagner's reagents. These qualitative tests indicate the presence or relative abundance of phytochemical classes but do not establish the identity of individual alkaloids present in the fraction.

Acute Oral Toxicity Study (Lorke Procedure)

The acute oral toxicity study was conducted according to the Lorke classical method (Lorke, 1983). In Phase I, nine male Wistar rats were randomly divided into three groups of three animals each (n = 3); Group I received 10 mg/kg, Group II received 100 mg/kg, and Group III received 1000 mg/kg body weight of the alkaloid-rich fraction, administered orally. Based on the absence of mortality in Phase I, Phase II was conducted using one rat per dose (n = 1), receiving 1500, 3000 and 5000 mg/kg, respectively, in accordance with the Lorke design for dose-range/mortality finding at higher doses. All animals in both phases were observed during the first 30 min after administration, periodically during the first 24 h, and daily for 14 days for signs of toxicity and mortality. Behavioural and physical changes, including tremors, salivation, convulsions, diarrhoea, lethargy, respiratory distress, piloerection, feeding behaviour, and locomotor activity, were monitored and recorded. The median lethal dose (LD_{50}) was estimated according to Lorke's method using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where: D_0 = highest dose producing no mortality; D_{100} = lowest dose producing mortality.

Toxicological Evaluation Group

A separate group of male Wistar rats (n = 3 per group), comprising a vehicle-treated control group and three treatment groups (1000, 3000 and 5000 mg/kg), was administered the alkaloid-rich fraction orally once and observed for 14 days under conditions identical to those described above. This group provided the animals used for body-weight, relative organ-weight, biochemical and histopathological evaluation described below.

Body and Relative Organ Weight Analysis

Body weights of animals in the toxicological evaluation group were recorded before treatment and on days 7 and 14 using a calibrated digital balance, and percentage weight change was calculated relative to baseline. At the end of the 14-day observation period, animals were humanely euthanised, and the liver, kidney, heart, lungs and spleen were excised, rinsed in ice-cold normal saline, blotted free of adherent fluid, and weighed immediately. Relative organ weight was expressed as grams of organ per 100 g of final body weight and computed using:

Relative Organ Weight (g/100 g body weight) = (Organ weight / Final body weight) × 100

Biochemical Analysis

Blood was collected by cardiac puncture into plain sample bottles, allowed to clot, and centrifuged at 3000 rpm for 10 min to obtain serum. Hepatic function biomarkers (ALT, AST, ALP, total bilirubin) and renal function biomarkers (urea, creatinine, cystatin C) were assessed using commercial diagnostic kits [manufacturer and catalogue numbers to be inserted by the authors], following the manufacturer's protocols and established clinical chemistry procedures (Tietz, 1995). ALT and AST were determined by the kinetic IFCC-referenced method, ALP by the p-nitrophenyl phosphate kinetic method, total bilirubin by the diazo method, urea by the modified Berthelot (urease) method, creatinine by the kinetic Jaffé method, and cystatin C by a particle-enhanced immunoturbidimetric assay. Intra-assay quality control was performed according to the kit manufacturer's recommendations for each run.

Histopathological Examination

At the end of the 14-day observation period, animals in the toxicological evaluation group were humanely euthanised under mild chloroform anaesthesia administered in accordance with the approved ethics protocol (BUK/ACUREC/CAP/PG223). Liver and kidney tissues were fixed in 10% buffered formalin for 48 h and processed by conventional histological technique (Bancroft & Gamble, 2008), comprising graded ethanol dehydration, xylene clearing, paraffin embedding, sectioning at 5 µm, and staining with haematoxylin and eosin (H&E). One representative section per organ per animal was examined by light microscopy (×100) by a histopathologist blinded to treatment-group identity, for evidence of necrosis, inflammatory cell infiltration, vascular congestion, cellular degeneration and architectural distortion.

Statistical Analysis

Data from the toxicological evaluation group are expressed as mean ± standard deviation (SD). Inter-group comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism version 9.0 (GraphPad Software Inc., USA); a value of $p < 0.05$ was considered statistically significant. Because Phase II of the Lorke procedure (1500, 3000 and 5000 mg/kg) used a single animal per dose, these observations are reported descriptively without variance statistics or significance testing (Table 3). Throughout, statistical significance was interpreted in conjunction with, and not independently of, the corresponding histopathological findings, consistent with best practice for integrated toxicological risk assessment.

RESULTS AND DISCUSSION

Extraction Yield

Sequential extraction and acid-base fractionation of the stem bark of *N. diderrichii* yielded a dark-brown alkaloid-rich fraction with a characteristic pungent odour. From 600 g of powdered stem bark, 198.34 g of crude methanolic extract was obtained (33.06% yield), from which 26.40 g of alkaloid-rich fraction was subsequently isolated, corresponding to 4.40% of the starting plant material (Table 1).

Table 1: % Yield of The Crude Extract and Alkaloid-Rich Fraction of *N. diderrichii* Stem Bark

Extract/Fraction	Weight (g)	Yield (%)
Powdered stem bark	600.00	—
Crude methanolic extract	198.34	33.06
Alkaloid-rich fraction	26.40	4.40

Phytochemical Screening

Qualitative screening confirmed strong enrichment of alkaloids in the fraction, consistent with the partitioning protocol employed, alongside moderate flavonoid and weak saponin content; terpenoids were not detected (Table 2).

Table 2: Phytochemical Constituents of the Alkaloid-Rich Fraction

Phytochemical	Observation	Inference
Alkaloids (Dragendorff's test)	Orange precipitate	Strongly present
Alkaloids (Wagner's test)	Brown precipitate	Strongly present
Flavonoids	Yellow colouration	Moderately present
Saponins	Persistent frothing	Slightly present
Terpenoids	No visible change	Absent

Acute Oral Toxicity and Clinical Observations (Lorke Procedure)

No mortality was recorded at any dose in either phase of the Lorke procedure, including the highest dose administered (5000 mg/kg, n = 1), yielding an estimated oral LD_{50} greater than 5000 mg/kg body weight. Clinical signs were dose-dependent but mild and self-limiting: no visible abnormality was observed at 10–100 mg/kg, while mild lethargy, transient piloerection and reduced locomotor activity emerged from 1000 mg/kg upward and intensified modestly with dose, without progression to convulsions, respiratory distress, diarrhoea or coma at any dose level (Table 3).

Table 3: Acute Oral Toxicity Observations Following Administration of the Alkaloid-Rich Fraction (Lorke Procedure)

Parameter	10 (n=3)	100 (n=3)	1000 (n=3)	1500 (n=1)	3000 (n=1)	5000 (n=1)
Mortality (dead/treated)	0/3	0/3	0/3	0/1	0/1	0/1
Behavioural change	None	Mild reduction in locomotion	Mild lethargy, piloerection	Slight reduction in locomotion	Mild lethargy, piloerection	Moderate lethargy, transient feed reduction

Dose in mg/kg Body Weight. Phase I Doses (10, 100, 1000 mg/kg) Tested n = 3 Per Group; Phase II Doses (1500, 3000, 5000 mg/kg) Tested n = 1 Per Dose as Prescribed by the Lorke Dose-Range-Finding Procedure, and are Reported Descriptively.

Body Weight Changes

In the toxicological evaluation group (n = 3 per group), body weight increased progressively across all groups over the 14 days. Weight gain in the 3000 and 5000 mg/kg groups was significantly lower than in the control group by day 14 ($p < 0.05$), while the 1000 mg/kg group did not differ significantly from control (Table 4).

Table 4: Body Weight Changes in Wistar Rats Treated with the Alkaloid-Rich Fraction (n = 3 Per Group)

Group	Initial weight (g)	Day 7 (g)	Day 14 (g)
Control	186.4 ± 4.7	194.8 ± 5.3	203.6 ± 5.9
1000 mg/kg	188.2 ± 5.1	194.1 ± 5.5	200.5 ± 6.1
3000 mg/kg	187.6 ± 4.9	191.8 ± 5.0	196.4 ± 5.4[a]
5000 mg/kg	189.1 ± 5.3	191.3 ± 5.2	194.7 ± 5.8[a]

Values are Mean ± SD (n = 3 Per Group). ^a^p < 0.05 vs Control (One-Way ANOVA, Tukey's Post Hoc Test).

Relative Organ Weights

Relative liver and kidney weights were significantly, though modestly, elevated at 3000 and 5000 mg/kg relative to control ($p < 0.05$), whereas heart, lung and spleen weights were unaffected at any dose (Table 5). No accompanying histopathological lesion was observed in liver or kidney at any dose.

Table 5: Relative Organ Weights (G/100 G Body Weight) of Wistar Rats Treated with the Alkaloid-Rich Fraction (n = 3 Per Group)

Organ	Control	1000 mg/kg	3000 mg/kg	5000 mg/kg
Liver	3.76 ± 0.18	3.94 ± 0.20	4.21 ± 0.25[a]	4.58 ± 0.31[a]
Kidney	0.69 ± 0.04	0.73 ± 0.05	0.81 ± 0.06[a]	0.89 ± 0.07[a]
Heart	0.41 ± 0.03	0.42 ± 0.03	0.44 ± 0.04	0.45 ± 0.03
Lungs	0.67 ± 0.05	0.69 ± 0.05	0.71 ± 0.06	0.73 ± 0.05
Spleen	0.38 ± 0.02	0.39 ± 0.03	0.41 ± 0.03	0.43 ± 0.04

Values are Mean ± SD, Expressed as g Organ Weight Per 100 g Final Body Weight (n = 3 Per Group). ^a^p < 0.05 vs Control (One-Way ANOVA, Tukey's Post Hoc Test).

Effect on Hepatic and Renal Function Biomarkers

Serum ALT, AST, ALP and total bilirubin increased in a dose-dependent manner, reaching statistical significance at 3000 and 5000 mg/kg relative to control ($p < 0.05$); the 1000 mg/kg group did not differ significantly from control for any hepatic parameter. A parallel pattern was observed for renal indices, with urea, creatinine and cystatin C significantly elevated at 3000 and 5000 mg/kg but not at 1000 mg/kg (Table 6). All values at the highest dose remained within a two-fold range of control means; interpretation of this

magnitude of change relative to the absence of histological injury is addressed in the discussion.

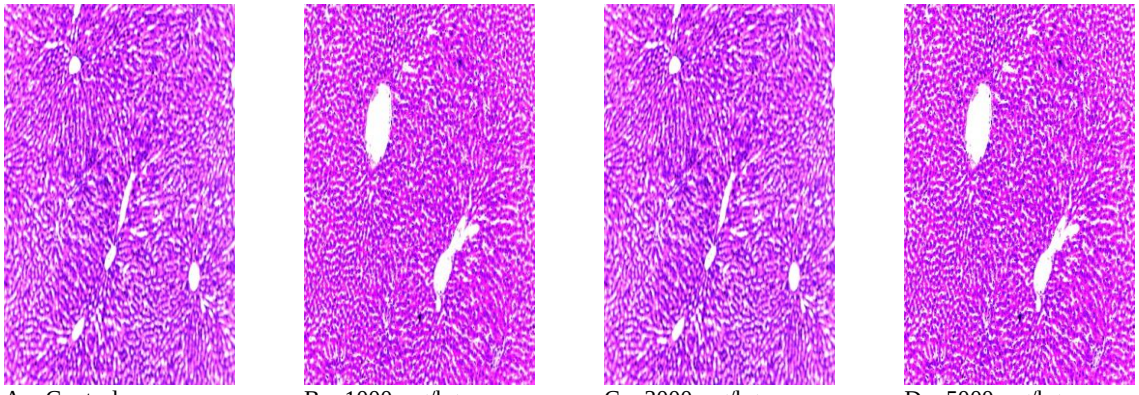
Table 6: Effect of the Alkaloid-Rich Fraction on Hepatic and Renal Function Biomarkers (n = 3 Per Group)

Parameter	Control	1000 mg/kg	3000 mg/kg	5000 mg/kg
ALT (U/L)	27.6 ± 2.9	34.8 ± 3.7	48.5 ± 4.6^{b}	63.7 ± 5.2^{b}
AST (U/L)	69.3 ± 5.1	77.4 ± 5.8	96.8 ± 7.4^{b}	118.5 ± 9.1^{b}
ALP (U/L)	86.7 ± 6.3	95.6 ± 7.1	123.4 ± 9.6^{b}	146.2 ± 11.3^{b}
Total bilirubin (mg/dL)	0.58 ± 0.05	0.71 ± 0.06	0.96 ± 0.08^{b}	1.29 ± 0.11^{b}
Urea (mg/dL)	28.6 ± 2.8	33.2 ± 3.0	44.9 ± 4.1^{b}	56.7 ± 5.2^{b}
Creatinine (mg/dL)	0.69 ± 0.05	0.82 ± 0.07	1.11 ± 0.08^{b}	1.43 ± 0.12^{b}
Cystatin C (mg/dL)	0.62 ± 0.04	0.74 ± 0.06	1.01 ± 0.08^{b}	1.36 ± 0.11^{b}

Values are Mean ± SD (n = 3 Per Group). ^b^p < 0.05 vs Control (One-Way ANOVA, Tukey's Post Hoc Test).

Histopathological Findings

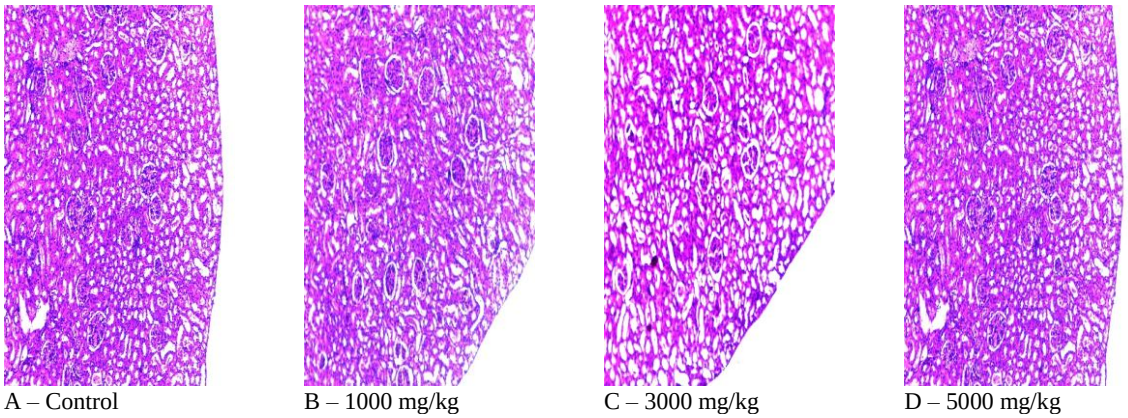
Photomicrographs of liver sections from the toxicological evaluation group revealed uniformly preserved hepatic architecture across all groups, characterised by intact hepatocytes arranged in well-defined cords radiating from unremarkable central veins. No necrosis, cytoplasmic vacuolation, inflammatory cell infiltration, sinusoidal congestion, or fibrotic change was observed at any dose, including 5000 mg/kg (Figure 1).



A – Control B – 1000 mg/kg C – 3000 mg/kg D – 5000 mg/kg

Figure 1: Histopathological Sections of Liver Tissue from Wistar Rats Treated with the alkaloid-Rich Fraction from the Stem Bark of *Nauclea diderrichii* (H&E Stain; ×100 Magnification)

Correspondingly, kidney sections from all groups showed normal glomerular and tubular architecture, with intact Bowman's capsules, unobstructed tubular lumina, and no evidence of tubular epithelial necrosis, interstitial inflammation, or glomerular collapse at any dose (Figure 2). The concordant absence of structural lesions in both organs across the entire dose range, despite the statistically significant biomarker elevations described above, constitutes the central finding of this study and is examined further in the discussion.



A – Control B – 1000 mg/kg C – 3000 mg/kg D – 5000 mg/kg

Figure 2: Histopathological Sections of Kidney Tissue from Wistar Rats Treated with the Alkaloid-Rich Fraction from the Stem Bark of *Nauclea diderrichii* (H&E Stain; ×100 Magnification)

Discussion

The absence of mortality up to 5000 mg/kg body weight placed the alkaloid-rich fraction of *N. diderrichii* stem bark in Category 6 (LD_{50} > 5000 mg/kg) of the Globally Harmonised System of hazard classification. The present finding is consistent with the wide safety margins previously reported for related *Nauclea* extracts and fractions (Akunne *et al.*, 2017; Douho Djimeli *et al.*, 2022).

The modest, dose-dependent increases in relative liver and kidney weight observed at 3000 and 5000 mg/kg, occurring without any accompanying necrosis, inflammation, or architectural distortion on histology, may plausibly reflect hypertrophic rather than pathological enlargement. This interpretation would be consistent with the consensus of the 3rd International ESTP Expert Workshop, which concluded that hepatomegaly driven by hepatocellular hypertrophy can be classified as an adaptive, non-adverse response when unaccompanied by corresponding histopathological or clinical-chemistry evidence of injury (Hall *et al*., 2012), though confirmation would require morphometric or ultrastructural evidence beyond the scope of the present study. A comparable compensatory mechanism, potentially involving increased tubular and glomerular workload, is one possible explanation for the parallel, modest increase in relative kidney weight, though nephron loss cannot be entirely excluded on routine H&E histology alone. The concurrent, modest attenuation of body-weight gain at these doses is consistent with transient, dose-related suppression of feed intake, given its resolution alongside the transient behavioural changes described in Table 3.

The central observation of this study is that statistically significant, dose-dependent elevations in ALT, AST, ALP, bilirubin, urea, creatinine and cystatin C at 3000 and 5000 mg/kg occurred without any corresponding structural lesion in liver or kidney tissue. This pattern should not be interpreted as an absence of biological effect; the elevated biomarkers are real biological effects that cannot be dismissed simply because routine H&E histology did not demonstrate lesions. Rather, it reflects the distinct and only partially overlapping information captured by biochemical and histological endpoints. Serum aminotransferases and alkaline phosphatase are sensitive to transient changes in hepatocellular membrane permeability and to metabolic/enzyme-induction responses mounted during xenobiotic biotransformation, both of which can elevate circulating enzyme activity well before, or even in the complete absence of, irreversible cell death (Messina and Duclos-Vallée, 2023; Thakur *et al.*, 2024). A directly analogous pattern, dose-dependent biomarker elevation with minimal or no accompanying histopathological injury, has been documented for other xenobiotics at comparably high exposure levels, reinforcing that this dissociation is a recognised, mechanistically plausible toxicological phenomenon rather than an anomaly specific to this fraction (Martins *et al*., 2024; Jimoh *et al*., 2026).

A similar argument applies to the renal indices. Serum urea, creatinine and cystatin C are functional markers of glomerular filtration and are influenced by haemodynamic and metabolic factors, including transient renal vasoconstriction, altered protein catabolism, and mild dehydration secondary to reduced feed and water intake at the highest doses, independent of any fixed structural nephron loss (Pottel *et al.*, 2024). Contemporary nephrotoxicology increasingly distinguishes these functional indices from structural tubular injury markers such as kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL), which can detect proximal tubular damage earlier, and with greater specificity, than creatinine-based indices (Yousef, 2025). As these structural biomarkers were not measured in the present study, the possibility of sub-histological tubular stress beneath the resolution of light microscopy cannot be entirely excluded, and this is identified as a limitation to be addressed in follow-up work. Nonetheless, the magnitude of biomarker change observed here, remaining within an approximately two-fold range of control values, together with the complete absence of histological injury even at the highest dose, is more consistent with reversible functional perturbation than with established structural hepatorenal toxicity (Hamad *et al*., 2026).

The wide acute safety margin and mild, reversible clinical profile observed here are broadly consistent with prior toxicological characterisation of *Nauclea* extracts, including reports of genotoxic and clastogenic activity of saponins extracted from *Nauclea* bark (Liu *et al*., 2011), the root bark methanol-dichloromethane extract evaluated by Akunne *et al.* (2017), and the aqueous and ethanolic stem bark extracts studied by Douho Djimeli *et al.* (2022), which similarly reported IC_{50} and oral LD_{50} values within conventional toxicity thresholds. Taken together with the phytochemical profile of the genus, dominated by indole alkaloids and phenolic constituents with reported antimalarial, antimicrobial, antiplasmodial and anti-inflammatory activity (Mamede *et al*., 2020; Omar *et al*., 2021; Bekoe *et al*., 2024), the present findings support the pharmacological promise of *N. diderrichii* while reinforcing that safety evaluation must rest on the integration of multiple, mechanistically distinct endpoints rather than any single indicator in isolation.

Despite these foundational toxicological findings in this study, it also has important limitations. First, qualitative phytochemical screening indicates the presence and relative abundance of chemical classes but does not establish the concentration or identity of individual alkaloids responsible for the observed effects; LC-MS/GC-MS-based characterisation is required to address this. Second, the 14-day observation window, while appropriate for acute toxicity characterisation, cannot exclude delayed or cumulative effects that might emerge on repeated dosing; a properly powered sub-acute study conducted in accordance with OECD Test Guideline 407 is recommended as the logical next step. Future work should additionally incorporate sensitive, structurally specific biomarkers of tubular and hepatocellular injury (e.g., KIM-1, NGAL, and oxidative stress indices) to more definitively establish the mechanistic basis of the biomarker–histopathology dissociation reported here.

CONCLUSION

The alkaloid-rich fraction of *N. diderrichii* stem bark caused no observed mortality up to the highest tested dose (5000 mg/kg) under the reported acute conditions, while higher doses (3000 and 5000 mg/kg) were associated with mild behavioural changes, reduced body-weight gain, elevated relative liver and kidney weight, and dose-dependent increases in hepatic and renal biochemical markers. Routine H&E histology did not reveal corresponding structural liver or kidney lesions at any dose. These findings should not be taken to indicate that the fraction is entirely safe, nor that the elevated biochemical markers are definitively non-adverse; rather, they suggest low acute lethality alongside evidence of dose-dependent biological effects at high exposure levels. Properly powered repeated-dose toxicity studies, quantitative alkaloid characterisation, and the inclusion of more sensitive, structurally specific organ-injury biomarkers are warranted before conclusions regarding the safety of this fraction, or of traditional preparations of *N. diderrichii* more broadly, can be drawn.

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CONFLICT OF INTEREST

The authors declare that they have no known competing interests that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article; raw experimental datasets are available from the corresponding author on reasonable request.

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