



Research Article

Phytochemical Composition and Safety Evaluation of Aqueous and Methanolic Leaf Extracts of African grape (*Lannea microcarpa*) in Swiss Albino Mice

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ABSTRACT

African grape (*Lannea microcarpa*) is widely used in West African ethnoveterinary medicine for treating livestock diseases, including trypanosomiasis. However, comprehensive safety data on its leaf extracts are limited. This study evaluated the phytochemical composition and safety profile of aqueous and methanolic leaf extracts of *L. microcarpa* in mice. Fresh leaves were extracted using water and methanol. Phytochemical screening was performed using standard qualitative and quantitative methods. Acute toxicity was assessed following OECD Guideline 423 at doses of 1000, 3000, and 5000 mg/kg body weight. Sub-acute toxicity was evaluated over 21 days following OECD Guideline 407, including body weight gain, relative organ weights, serum biochemical parameters (hepatic and renal function), and histopathological examination of liver and kidney. Both extracts contained alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, steroids, and cardiac glycosides. Methanolic extraction yielded significantly higher total phenolics (86.42 ± 6.31 mg GAE/g), terpenoids (72.54 ± 2.16 mg/g), and steroids (31.42 ± 1.27 mg/g), while aqueous extraction yielded higher flavonoids (98.34 ± 3.67 mg QE/g) and saponins (69.34 ± 2.18 mg/g). The oral LD₅₀ of both extracts exceeded 5000 mg/kg body weight. Sub-acute administration produced no significant changes ($p > 0.05$) in body weight gain, relative organ weights, or serum biochemical parameters. Histopathological examination showed only mild, non-significant changes at higher doses. African grape (*Lannea microcarpa*) leaf extracts are rich in bioactive phytochemicals and possess a high safety margin, with LD₅₀ > 5000 mg/kg. Repeated oral administration for 21 days caused no significant hepatotoxicity or nephrotoxicity, supporting their safety for medicinal use.

Keywords: Acute toxicity; African grape phytochemicals; Histopathology; LD₅₀; Safety evaluation; Sub-acute toxicity

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INTRODUCTION

African trypanosomiasis, a neglected tropical disease caused by pathogenic flagellated protozoa belonging to the genus *Trypanosoma*, continues to pose severe public health and economic challenges across sub-Saharan Africa (Büscher *et al.*, 2017; Giordani *et al.*, 2016). The disease manifests primarily in two forms: Human African Trypanosomiasis (HAT, or sleeping sickness) and Animal African Trypanosomiasis (AAT, or Nagana) (Giordani *et al.*, 2016). In livestock, AAT represents a major biological obstacle to sustainable

agricultural productivity, food security, and rural poverty alleviation (Auty *et al.*, 2021). Endemic across approximately 10 million square kilometers spanning 37 sub-Saharan African nations, AAT directly compromises livestock production within the continent's tsetse belt, putting an estimated 50 million head of cattle and 230 million small ruminants at risk of infection (Auty *et al.*, 2021; Giordani *et al.*, 2016). Clinical manifestations in susceptible animals include progressive anemia, intermittent pyrexia, severe emaciation, lymphadenopathy, immunosuppression, and reproductive failure (Atata

et al., 2024; Giordani *et al.*, 2016). Consequently, AAT accounts for direct annual economic losses of USD 1.0 to 1.2 billion in animal production, with indirect agricultural losses exceeding USD 4.5 billion due to the loss of draft animal power needed for crop cultivation (Auty *et al.*, 2021).

Control strategies for AAT rely largely on vector management and veterinary chemotherapy (Auty *et al.*, 2021). However, large-scale tsetse fly suppression remains logistically demanding, expensive, and vulnerable to vector re-invasion (Giordani *et al.*, 2016). As a result, disease management depends heavily on a small panel of synthetic trypanocides registered over six decades ago, principally diminazene acetate, isometamidium chloride, and homidium salts (Ibrahim *et al.*, 2014; Shehu *et al.*, 2024). The continuous and unmonitored use of these legacy compounds has led to drug resistance across at least 21 African nations (Giordani *et al.*, 2016). In Nigeria, field isolates of *Trypanosoma brucei brucei* exhibit resistance to standard doses of diminazene acetate and isometamidium chloride, resulting in post-treatment relapse rates of 40% to 60% in experimental models (Ibrahim *et al.*, 2014; Shehu *et al.*, 2024). Furthermore, synthetic trypanocides possess narrow therapeutic margins; diminazene acetate, for example, induces severe hepato-renal impairment and central nervous system damage at doses slightly above therapeutic levels (Giordani *et al.*, 2016; Shehu *et al.*, 2024).

These chemotherapeutic limitations have driven interest toward medicinal plants as sources of novel bioactive secondary metabolites with antiparasitic potential (Ibrahim *et al.*, 2014; Ouedraogo *et al.*, 2022). Over 60% of rural livestock farmers in sub-Saharan Africa depend on ethnoveterinary remedies to treat animal diseases (Traore *et al.*, 2022). Among West African flora, African grape (*Lannea microcarpa*) Engl. & K. Krause (family Anacardiaceae), commonly known as the African grape tree or *Kuntunkuni*, features prominently in ethnoveterinary medicine (Belemnaba *et al.*, 2020; Nitiéma *et al.*, 2023). Leaf decoctions of *L. microcarpa* are widely used by pastoralists and traditional healers across West Africa to treat livestock fevers, gastrointestinal disorders, helminthiasis, and suspected trypanosomiasis (Belemnaba *et al.*, 2020; Traore *et al.*, 2022).

Phytochemical characterization has shown that *L. microcarpa* leaves contain diverse bioactive polyphenols and flavonoids, such as gallic acid, epicatechin, vitexin, and myricetin derivatives (Kinda *et al.*, 2021; Ouedraogo *et al.*, 2022). Pharmacological

evaluations have demonstrated that *L. microcarpa* extracts exhibit strong antioxidant, anti-inflammatory, antibacterial, and preliminary antiparasitic properties (Ilboudo *et al.*, 2023; Nitiéma *et al.*, 2023). However, comprehensive safety data regarding its leaf extracts remain limited (Ilboudo *et al.*, 2023). Most biological studies on this species have relied on *in vitro* models, which do not reflect host physiological processes such as oral bio-absorption, hepatic metabolic transformation, plasma protein binding, organ accumulation, or systemic clearance in a living mammalian host (Kinda *et al.*, 2021; Shehu *et al.*, 2024). Furthermore, solvent polarity influences phytochemical extraction: aqueous preparations mirror traditional pastoral decoctions, whereas alcoholic solvents extract broader polar and semi-polar secondary metabolite profiles (Nitiéma *et al.*, 2023; Ouedraogo *et al.*, 2022).

Therefore, this study evaluated the phytochemical composition, acute toxicity (LD₅₀), and 21-day sub-acute toxicity profiles of aqueous and methanolic leaf extracts of *L. microcarpa* on body weight gain, organ weights, serum biomarkers of hepatic and renal function, and liver and kidney histopathology in Swiss albino mice. The study predicted that there is no significant difference in the safety profiles of the aqueous and methanolic leaf extracts of *L. microcarpa*, and that both extracts would produce no significant toxicity in mice following acute and repeated oral administration.

MATERIALS AND METHODS

Study Area

The study was conducted at the Department of Biology, Ibrahim Badamasi Babangida University, Lapai, Niger State. Locate at 9°05'07.22" N, and 6°33'49.53" E. All experimental procedures were conducted at the Mini Garden of the Department of Animal Biology, Ibrahim Badamasi Babangida University (IBBUL), Lapai, Niger State, Nigeria. The Mini Garden is a designated research facility within the university campus used for plant cultivation and maintenance of experimental animals under environmental conditions. The university is geographically located between latitudes 9°03'17.60" N and 9°05'07.22" N, and longitudes 6°33'49.53" E and 6°35'38.47" E. The main campus is situated at 9°04'03" N, 6°34'11" E (9.06738° N, 6.56979° E), with an elevation of about 169 metres above sea level. The facility is located along the Lapai–Minna Road, approximately 3 kilometres from Lapai Town.

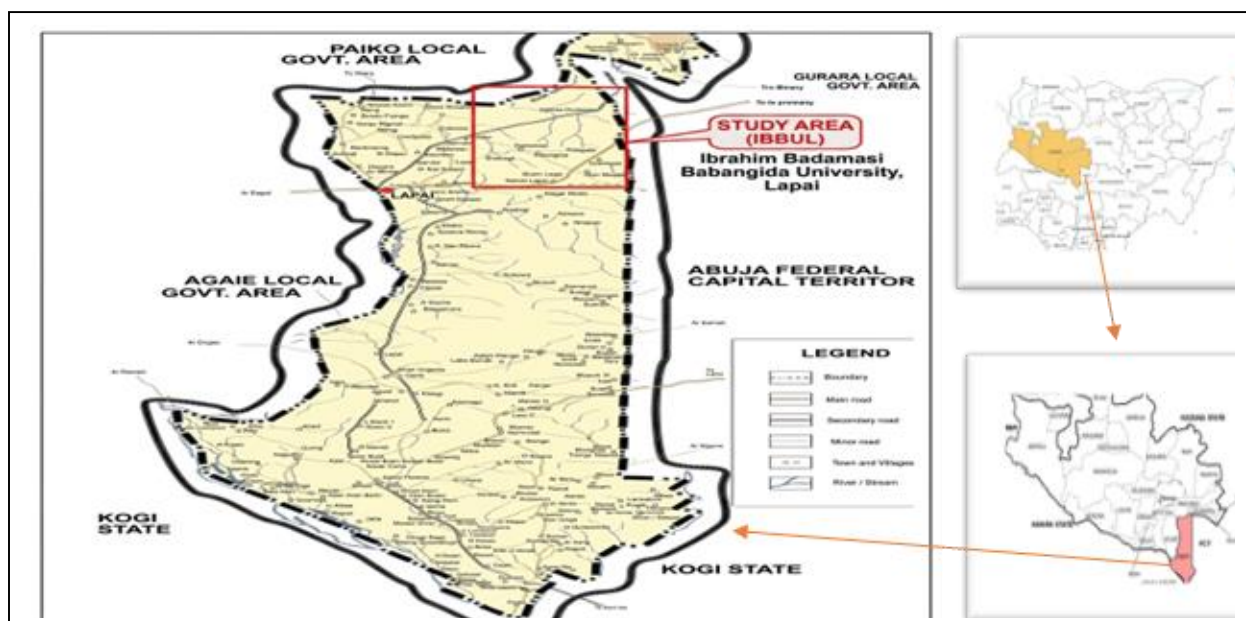


Fig 1: Map of Lapai showing the study area

Research Design

The study adopted an experimental, randomized, controlled laboratory design. It was conducted in five phases: (i) collection, authentication, and preparation of plant extracts; (ii) qualitative and quantitative phytochemical screening; (iii) acute toxicity evaluation; (iv) sub-acute toxicity evaluation; and (v) in vivo Anti-trypanosomal efficacy evaluation.

Collection and Authentication of Plant Material

Apparently healthy, fresh, mature leaves of African grape were collected at IBBUL farm site. A representative sample of the collected plant material was taken to the Department of Biology, IBB University Lapai, for formal identification. The plant was identified with ID No. IBBU/FNS/0013.

Preparation of Plant Extracts

The fresh leaves of African grape were sorted to remove damaged leaves and foreign materials. They were washed thoroughly under running tap water, followed by rinsing with distilled water. The washed leaves were spread in a single layer on clean laboratory paper and shade-dried at room temperature for 7 to 10 days until brittle (Harborne, 1998). The dried leaves were ground into a coarse powder using an electric blender and was passed through a 40-mesh sieve to obtain a uniform particle size. The powdered leaves were stored in airtight amber glass containers at room temperature until extraction (Evans, 2009).

Aqueous Extraction

One hundred grams (100 g) of the powdered leaves were dissolved in 1,000 mL of distilled water in a conical flask (Azwanida, 2015). The mixture was kept at room temperature for 24 hours with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C. The concentrated extract was further dried on a water bath at 25°C to remove most of the solvents from the extracts (Tiwari *et al.*, 2011). The dried aqueous extract was weighed, and the percentage yield was calculated. The extract was stored in an airtight container at 4°C until use (Sasidharan *et al.*, 2011).

The percentage yield of the aqueous extract was calculated using the formula:

$$\text{Percentage Yield (\%)} = \left(\frac{\text{Weight of Dried Extract}}{\text{Weight of Powdered Leaves}} \right) \times 100$$

The percentage yield of the aqueous extract was 12.40% (w/w).

Methanolic Extraction

One hundred grams (100 g) of the powdered leaves were dissolved in 1,000 mL of methanol in a conical flask (Azwanida, 2015). The mixture was kept at room temperature for 48 hours with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C. The concentrated extract was further dried on a water bath at 25°C to remove most

of the solvents from the extracts (Tiwari *et al.*, 2011). The dried methanolic extract was weighed, and the percentage yield was calculated. The extract was stored in an airtight container at 4°C until use (Sasidharan *et al.*, 2011).

The percentage yield of the methanolic extract was calculated using the formula:

$$\text{Percentage Yield (\%)} = \left(\frac{\text{Weight of Dried Extract}}{\text{Weight of Powdered Leaves}} \right) \times 100$$

The percentage yield of the methanolic extract was 18.10% (w/w).

Preliminary Phytochemical Screening of Extracts

The aqueous and methanolic leaf extracts of African grape (*Lannea microcarpa*) were subjected to preliminary phytochemical screening to identify the presence of secondary metabolites (alkaloids, flavonoids, tannins, saponins, phenolic compound, steroids and terpenes, and cardiac glycosides using standard qualitative and quantitative tests as described by Harborne (1998) and Morsy (2014). All tests were performed in triplicate (Morsy, 2014).

Quantitative Phytochemical Analysis

Quantitative analysis of the phytochemical constituents was conducted to determine the concentrations of saponins, flavonoids, tannins, terpenoids, steroids, and cardiac glycosides according to standard procedures as described by Harborne (1998) and Morsy (2014). The results were expressed as mean ± standard deviation.

Experimental Animals

Sixty (60) Swiss albino mice of both sexes (20–35 g, 4–5 weeks old) were obtained from the Animal House, Department of Biochemistry, IBBUL, Lapai, Niger State, Nigeria, and housed in polypropylene cages with stainless steel wire mesh covers and wood shavings bedding (changed every 48 hours) under standard laboratory conditions (room temperature; 12-hour light/dark cycle) (National Research Council, 2011; Wolfensohn & Lloyd, 2013). The mice were acclimatized for seven days with gentle daily handling, fed commercial rodent pellets and clean water *ad libitum*, and examined daily for signs of illness or distress (National Research Council, 2011; Wolfensohn & Lloyd, 2013).

Ethical Consideration

The experimental protocol was evaluated and officially approved by the Research Ethics Committee of Ibrahim Badamasi Babangida University (IBBUL), Lapai (Protocol Reference Number: IBBUL/12/2026). All interventions were humanely executed, and the study layout complied thoroughly with the standard

protocols of the Organization for Economic Co-operation and Development (OECD, 2001) for animal testing

Acute Toxicity Study (LD₅₀ Determination)

The acute toxicity of the aqueous and methanolic leaf extracts of African grape (*Lannea microcarpa*) was determined using the OECD 423 guidelines as described by the Organisation for Economic Co-operation and Development (OECD, 2001) and Lorke (1983).

Acute Toxicity Study (LD₅₀ Determination)

Mice were fasted overnight (12–16 hours) with water provided *ad libitum* before extract administration (OECD, 2001; Lorke, 1983). The mice were randomly divided into seven groups of four mice each (n = 4): Group 1 (control) received distilled water only; Groups 2–4 received aqueous extract at 1000, 3000, and 5000 mg/kg body weight; and Groups 5–7 received methanolic extract at 1000, 3000, and 5000 mg/kg body weight (Lorke, 1983). Extracts were administered orally using an oral gavage needle attached to a 1 mL syringe (OECD, 2001; Lorke, 1983). After administration, animals were observed continuously for 1 hour, intermittently for 4 hours, and once every 24 hours for three days for signs of acute toxicity, including dyspnea, lethargy, and other abnormal habits (OECD, 2001; Lorke, 1983). Mice were weighed before administration and at 24 and 48 hours post-administration (Lorke, 1983). If no mortality occurred at 5,000 mg/kg, the LD₅₀ was considered greater than 5,000 mg/kg and the extract classified as "practically non-toxic"; lower doses (100, 200, and 300 mg/kg) were selected for the main efficacy study (OECD, 2001; Lorke, 1983).

Sub-Acute Toxicity Study

The sub-acute toxicity study was conducted following the method described by Shehu *et al.* (2024) and Kifayatullah *et al.* (2015) to evaluate the safety of both extracts following repeated daily oral administration for 21 days. Twenty-eight (28) mice were randomly divided into seven groups of four mice each (n = 4): Group 1 (control) received distilled water only; Groups 2–4 received aqueous extract at 1000, 3000, and 5000 mg/kg body weight; and Groups 5–7 received methanolic extract at 1000, 3000, and 5000 mg/kg body weight (Shehu *et al.*, 2024). Extracts were administered orally via gavage needle once daily for 21 consecutive days at midday (12:00 hours) (Shehu *et al.*, 2024). Toxicity signs were recorded at intervals of 10 minutes, 30 minutes, 1 hour, 4 hours, 8 hours, and 24 hours after each dose, with daily observations for food intake, water intake, respiration, eye color, movement, convulsion, aggressiveness, sedation,

mortality, survival, and any other abnormal signs (Shehu *et al.*, 2024).

Serum Collection and Biochemical Analysis

Blood samples were collected into non-heparinized tubes, centrifuged at 3000 rpm for 10 minutes, and the serum was stored frozen for biochemical analysis (Shehu *et al.*, 2024). Hepatic enzymes (AST, ALT, ALP) were evaluated as described by Owolarafe *et al.* (2017) and Pieme *et al.* (2006), while renal function tests (urea, uric acid, creatinine, bilirubin, protein, and electrolytes) were evaluated using ready-to-use kits from Sigma Aldrich based on the method of Aniagu *et al.* (2005).

Body Weight Gain and Relative Organ Weight

Body weight gain was calculated as the difference between final and initial weights (Shehu *et al.*, 2024). Relative organ weight (ROW) was calculated using the formula: ROW = (Absolute Organ Weight [g] / Body Weight at Sacrifice [g]) × 100 (Shehu *et al.*, 2024; OECD, 2002).

Histopathological Examination

Liver and kidney tissues were stabilized in fixative, dehydrated, cleaned, impregnated with wax, and embedded in paraffin wax for block formation (Shehu *et al.*, 2024; Jn *et al.*, 2019). Blocks were trimmed, sectioned using a rotary microtome, fixed onto glass slides, and de-waxed on a water bath at 40°C (Zainal *et al.*, 2020). Slides were stained with iron hematoxylin and eosin (H&E), mounted on Canada balsam, and viewed under X10 and X40 objectives (Shehu *et al.*, 2024).

Data Analysis

All data were analyzed using SPSS version 26 (SPSS Inc., Chicago, IL, USA) and expressed as Mean ± Standard Deviation (SD). Descriptive statistics summarized the phytochemical data (n = 3), and LD₅₀ was calculated using Lorke's formula (Lorke, 1983). One-way Analysis of Variance (ANOVA) was used to compare means of body weight gain, relative organ weights, and serum biochemical parameters among treatment groups, followed by Tukey's Honest Significant Difference (HSD) post-hoc test for multiple comparisons (Shehu *et al.*, 2024).

RESULTS

Phytochemical Composition of Extracts

Qualitative screening revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, steroids, saponins, and cardiac glycosides in both extracts, with methanolic extraction yielding stronger reactions for terpenoids and steroids, while the aqueous extract showed higher amounts of flavonoids, saponins, and cardiac glycosides.

Quantitative analysis confirmed that total flavonoids were most abundant in the aqueous extract (98.34 ± 3.67 mg QE/g) while total phenolics were most abundant in the methanolic extract (86.42 ± 6.31 mg GAE/g). The aqueous extract contained significantly higher levels of flavonoids (98.34 ± 3.67 vs 78.85 ± 4.12 mg QE/g) and saponins (69.34 ± 2.18 vs 21.27 ± 1.85 mg/g) compared to the methanolic extract (p < 0.05). In contrast, methanolic extraction yielded significantly higher concentrations of terpenoids (72.54 ± 2.16 vs 26.44 ± 1.98 mg/g), steroids (31.42 ± 1.27 vs 22.41 ± 1.11 mg/g), and total phenolics (86.42 ± 6.31 vs 20 ± 5.28 mg GAE/g). Cardiac glycosides were higher in the methanolic extract (28.63 ± 0.84 vs 23.85 ± 0.62 mg/g), while tannins were higher in the aqueous extract (22.61 ± 2.41 vs 14.82 ± 2.16 mg TAE/g). Alkaloids were present in similar concentrations in both extracts (25.62 ± 1.54 mg/g aqueous vs 21.71 ± 1.83 mg/g methanolic), as shown in Table 1.

Acute Toxicity Profile of Aqueous and Methanolic Extract of African Grape in Mice

The acute oral toxicity study was evaluated following OECD Guideline 423. No mortality was recorded in any experimental mice administered either aqueous or methanolic extract at doses of 1000, 3000, or 5000 mg/kg body weight, and no severe clinical signs were observed throughout the 72-hour monitoring period (Table 2). At the highest dose (5000 mg/kg), mild transient hypoactivity and sedation were observed in three mice treated with methanolic extract and two mice receiving aqueous extract, which resolved completely within two hours. Since no mortality occurred at the limit dose of 5000 mg/kg, the median lethal dose (LD₅₀) of both extracts was considered to be greater than 5000 mg/kg body weight, classifying both extracts as practically non-toxic according to OECD toxicity classification.

Sub-Acute Toxicity Profile of Aqueous and Methanolic Extracts of African Grape in Mice

The sub-acute toxicity of the aqueous and methanolic leaf extracts of African grape (*Lannea microcarpa*) was evaluated following repeated daily oral administration for 21 days. The observations made during the study period are presented below.

Clinical Observations

No mortality was recorded in any of the experimental mice administered either aqueous or methanolic extract at doses of 1000, 3000, or 5000 mg/kg body weight throughout the 21-day observation period. Likewise, no severe clinical signs such as convulsion, tremors, salivation, respiratory distress, diarrhoea, or coma were observed. All animals remained active,

with normal grooming behaviour, feeding activity, water intake, respiration, and locomotion throughout the study period. No sedation or abnormal

movements were observed at any of the tested doses. Sub-acute study findings were summarized in Table 3.

Table 1: Qualitative and Quantitative Phytochemical Composition of *Lannea microcarpa* Leaf Extracts

Phytochemical	Aqueous Extract	Methanolic Extract
Alkaloids	+ (25.62 ± 1.54)	+ (21.71 ± 1.83)
Flavonoids	+ (98.34 ± 3.67)	+ (78.85 ± 4.12)
Total phenolics	+ (20 ± 5.28)	+ (86.42 ± 6.31)
Tannins	+ (22.61 ± 2.41)	+ (14.82 ± 2.16)
Saponins	+ (69.34 ± 2.18)	+ (21.27 ± 1.85)
Terpenoids	+ (26.44 ± 1.98)	+ (72.54 ± 2.16)
Steroids	+ (22.41 ± 1.11)	+ (31.42 ± 1.27)
Cardiac glycosides	+ (23.85 ± 0.62)	+ (28.63 ± 0.84)

Values are presented as Mean ± SD (n = 3).

Table 2: Acute Toxicity Profile (LD₅₀) of Aqueous and Methanolic Leaf Extracts of African grape in Mice

Observation	Control	AqE 1000	AqE 3000	AqE 5000	MeE 1000	MeE 3000	MeE 5000
Feeding	N	N	N	N	N	N	AbN
Water intake	N	N	N	N	N	N	N
Eye Color	N	N	N	N	N	N	N
Locomotion	N	N	N	AbN	N	N	AbN
Respiration	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N
Sedation	N	N	N	AbN	N	N	AbN
Recovery	;	Complete	Complete	Complete	Complete	Complete	Complete
Mortality	0/4	0/4	0/4	0/4	0/4	0/4	0/4

Keys, AqE = Aqueous Extract; MeE = Methanolic Extract; N = Normal; AbN = Abnormal

LD₅₀ > 5000 mg/kg body weight.

Table 3: Sub-acute toxicity Profile of Aqueous and Methanol Leaf Extracts of African grape on Swiss albino mice for 21 days

Parameters	Control	AqE 1000	AqE 3000	AqE 5000	MeE 1000	MeE 3000	MeE 5000
Food intake	N	N	N	N	N	N	N
Water intake	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N
Eye Color	N	N	N	N	N	N	N
Movement	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N
Aggressiveness	N	N	N	N	N	N	N
Sedation	N	N	N	N	N	N	N
Death/Survive	S	S	S	S	S	S	S

Keys, AqE = Aqueous Extract; MeE = Methanolic Extract; N = Normal; AbN = Abnormal; S = Survived; D = Dead

Effects of Aqueous and Methanolic Leaf Extracts on Body Weight Gain and Relative Organ Weight

The effects of aqueous and methanolic leaf extracts on body weight gain and relative organ weights are presented in Table 4. There were no significant differences ($p > 0.05$) in the initial body weights of mice before treatment. All groups showed

progressive weight gain throughout the 21-day treatment period, with the highest body weight gain recorded in the control group (8.20 ± 0.84 g) and the lowest in groups treated with the highest dose (5000 mg/kg) of both extracts, although these differences were not statistically significant ($p > 0.05$). Similarly, no significant differences ($p > 0.05$) were observed in

the relative weights of liver, kidney, and spleen between the control and extract-treated groups, and all values remained within normal reference ranges for mice. These findings indicate that the extracts did not induce significant organ toxicity.

Effects of Aqueous and Methanolic Leaf Extracts of African Grape on Serum Biochemical Parameters of Mice

The effects of aqueous and methanolic leaf extracts on serum biochemical parameters for hepatic and renal function are presented in Figures 4.1 and 4.2 respectively. For hepatic function, the mean values of total protein, albumin, and bilirubin in the control group (72.8 ± 0.4 g/L, 34.6 ± 0.3 g/L, and 9.8 ± 0.2 μ mol/L, respectively) were slightly lower than those of groups treated with the highest doses of both extracts: AqE 5000 (total protein: 78.6 ± 0.5 g/L; albumin: 39.8 ± 0.4 g/L; bilirubin: 10.8 ± 0.3 μ mol/L) and MeE 5000 (total protein: 77.4 ± 0.6 g/L; albumin: 39.0 ± 0.3 g/L; bilirubin: 10.6 ± 0.2 μ mol/L). Similarly, the mean values of hepatic enzymes in the control group (ALT: 25.8 ± 0.3 U/L; AST: 165.4 ± 0.5 U/L; ALP: 92.6 ± 0.4 U/L) were lower than those of AqE 5000 (ALT: 29.6 ± 0.4 U/L; AST: 171.2 ± 0.6 U/L; ALP: 95.4 ± 0.3 U/L) and MeE 5000 (ALT: 30.2 ± 0.5 U/L; AST: 172.0 ± 0.7 U/L; ALP: 96.2 ± 0.4 U/L). However, these differences were not statistically significant ($p > 0.05$), and all values remained within normal reference ranges, indicating that the extracts did not induce hepatotoxicity.

For renal function, the mean values of sodium, potassium, chloride, urea, creatinine, and bicarbonate in the control group (134.6 ± 0.5 mmol/L, 4.6 ± 0.3 mmol/L, 94.8 ± 0.4 mmol/L, 3.4 ± 0.2 mmol/L, 88.6 ± 0.3 μ mol/L, and 24.8 ± 0.3 mmol/L, respectively) were slightly lower than those of groups treated with the highest doses of both extracts. For AqE 5000, the values were: sodium (139.2 ± 0.6 mmol/L), potassium (4.8 ± 0.2 mmol/L), chloride (96.8 ± 0.4 mmol/L), urea (7.0 ± 0.3 mmol/L), creatinine (97.8 ± 0.5 μ mol/L), and bicarbonate (27.2 ± 0.4

mmol/L). For MeE 5000, the values were: sodium (137.8 ± 0.4 mmol/L), potassium (4.9 ± 0.3 mmol/L), chloride (97.4 ± 0.5 mmol/L), urea (6.8 ± 0.4 mmol/L), creatinine (96.4 ± 0.6 μ mol/L), and bicarbonate (27.8 ± 0.5 mmol/L). There were no significant differences ($p > 0.05$) between the control and treated groups, and all values remained within the normal reference range, indicating that the extracts did not induce nephrotoxicity at the tested doses.

Histopathological Examination of Liver and Kidney of Mice Administered with Aqueous and Methanolic Extracts of African Grape

The histopathological examination of liver and kidney sections from mice treated with aqueous and methanolic leaf extracts of *Lannea microcarpa* for 21 days is summarized below. Liver sections from control mice showed normal hepatic architecture with well-preserved hepatocytes, normal central veins, sinusoids, and no inflammatory infiltrates or necrosis (Plate 1). Mice treated with 1000 mg/kg of both extracts showed normal hepatocellular architecture similar to the control (Plates 2 and 6). Mice treated with 3000 and 5000 mg/kg of both extracts showed mild histological changes, including mild dilation of sinusoids and mild disorganization of hepatic cords, but these changes were minimal and not considered toxicologically significant (Plates 3, 4, 7, and 8). No necrosis or severe inflammation was observed.

Kidney sections from control mice displayed normal glomeruli, intact Bowman's capsules, normal proximal and distal tubules, and no inflammatory infiltration (Plate 9). Mice treated with 1000 mg/kg of both extracts showed normal architecture of glomeruli and tubules similar to the control (Plates 10 and 13). Mice treated with 3000 and 5000 mg/kg of both extracts showed mild histological changes, including mild reduction of glomerulus cells and mild mesenchymal proliferation, but these changes were minimal and not considered toxicologically significant (Plates 11 and 12). No necrosis or severe inflammation was observed.

Table 4: Effects of Aqueous and Methanolic Leaf Extracts of African grape on Body Weight Gain and Relative Organ Weights of Mice for 21 Day

Group	Body Weight Gain (g)	Liver (%)	Kidney (%)	Spleen (%)
Control	8.20 ± 0.84^a	4.82 ± 0.31^a	1.12 ± 0.08^a	0.38 ± 0.05^a
AqE 1000 mg/kg	8.00 ± 0.76^a	4.78 ± 0.28^a	1.10 ± 0.07^a	0.37 ± 0.04^a
AqE 3000 mg/kg	7.70 ± 0.82^a	4.75 ± 0.25^a	1.08 ± 0.09^a	0.36 ± 0.06^a
AqE 5000 mg/kg	7.40 ± 0.78^a	4.70 ± 0.30^a	1.06 ± 0.06^a	0.35 ± 0.04^a
MeE 1000 mg/kg	7.90 ± 0.72^a	4.80 ± 0.27^a	1.11 ± 0.08^a	0.37 ± 0.05^a
MeE 3000 mg/kg	7.80 ± 0.85^a	4.76 ± 0.29^a	1.09 ± 0.07^a	0.36 ± 0.06^a
MeE 5000 mg/kg	7.40 ± 0.80^a	4.72 ± 0.26^a	1.07 ± 0.08^a	0.35 ± 0.05^a

Key: AqE = Aqueous Extract; MeE = Methanol Extract.

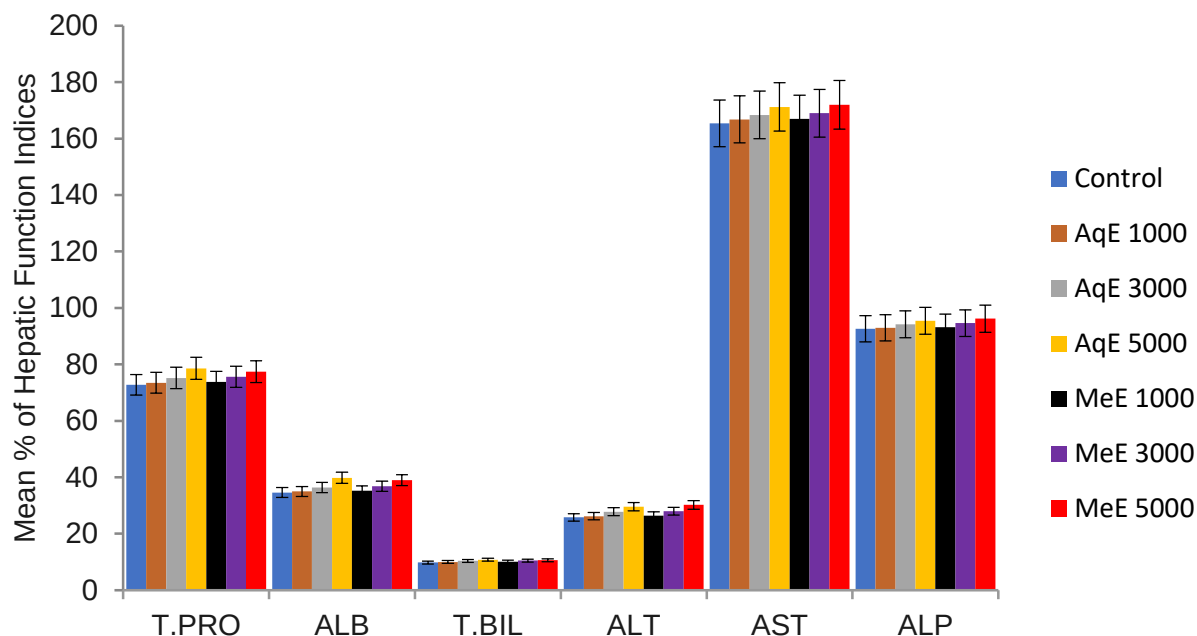


Figure 2: Effects of Aqueous and Methanolic Leaf Extracts of African Grape on Hepatic Serum Biochemical Parameters

Key: AqE (Aqueous Extract); MeE (Methanol Extract); T.Pro (Total protein); ALB (Albumin); T.Bil (Total bilirubin); ALT (Alanine transaminase); AST (Aspartate transaminase); ALP (Alkaline phosphatase).

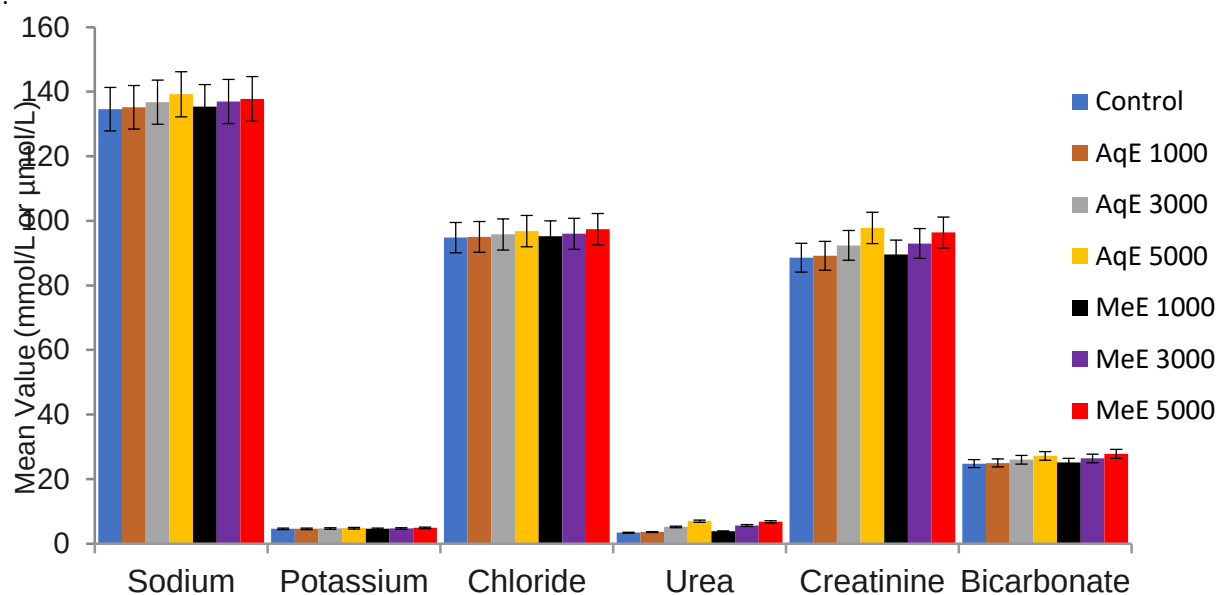


Figure 3: Effects of Aqueous and Methanolic Leaf Extracts of African Grape on Serum Biochemical Parameters (Renal Function) in Mice

Key: AqE (Aqueous Extract); MeE (Methanol Extract);



Plate 1. Photomicrograph of the liver section from the control mice showing normal hepatic architecture with well-arranged hepatocytes, intact hepatic cords, normal sinusoids, and a normal central vein (x40).

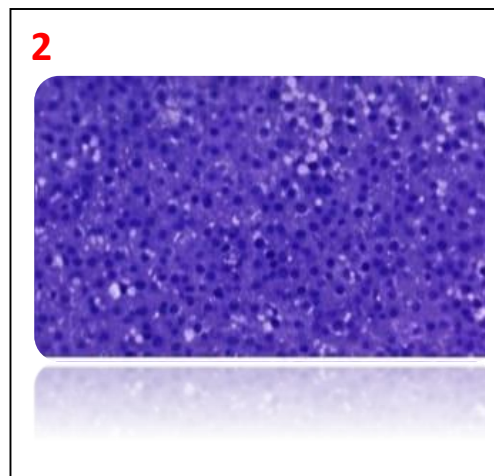


Plate 2. Photomicrograph of the liver section from mice treated with 1000 mg/kg aqueous extract showing preserved hepatic architecture with normal hepatocytes and no obvious pathological alterations (x40).

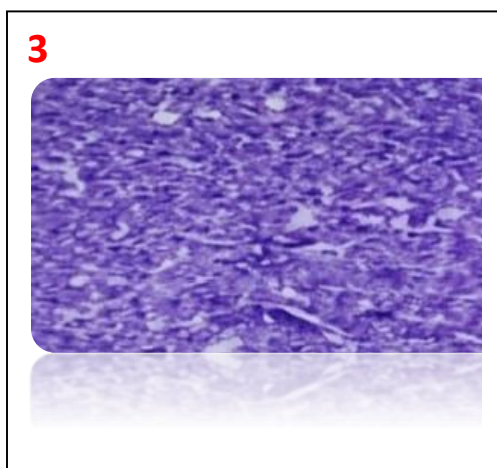


Plate 3. Photomicrograph of the liver section from mice treated with 3000 mg/kg aqueous extract showing mild hepatocellular degeneration with slight distortion of the hepatic architecture (x40).

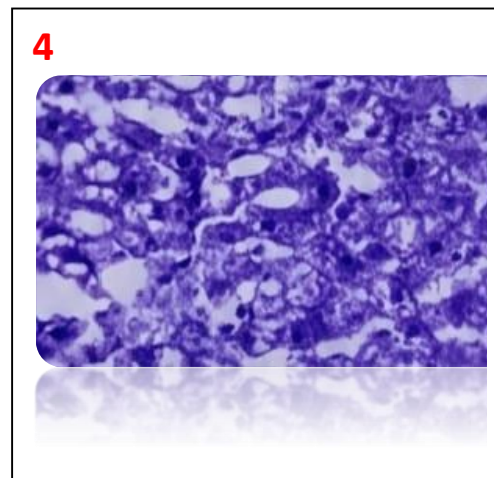


Plate 4. Photomicrograph of the liver section from mice treated with 5000 mg/kg aqueous extract showing mild vacuolar degeneration of hepatocytes with preserved overall hepatic architecture (x40)

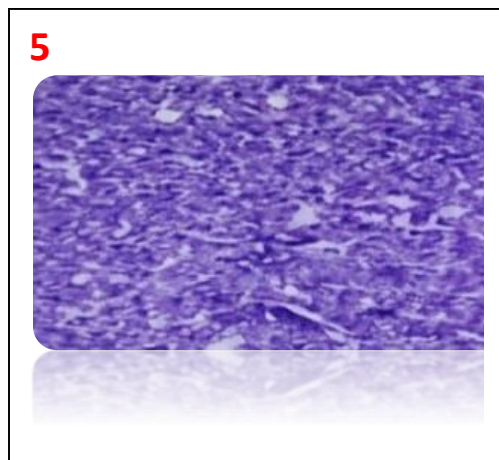


Plate 5. Photomicrograph of the kidney section from the control mice showing normal renal architecture with intact glomeruli, Bowman's capsules, renal tubules, and interstitial tissue ($\times 40$).



Plate 6. (Not provided.) Photomicrograph of the kidney section from mice treated with 1000 mg/kg aqueous extract showing preserved renal architecture with normal glomeruli and renal tubules ($\times 40$).

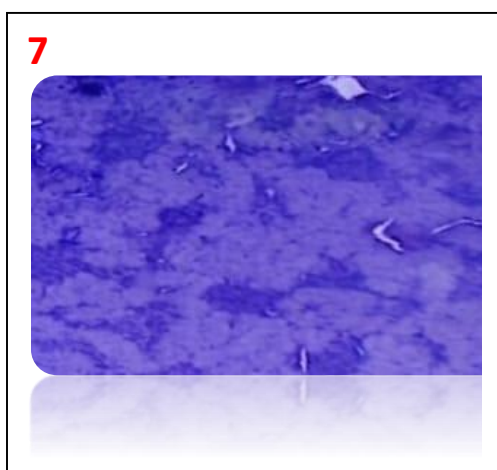


Plate 7. Photomicrograph of the kidney section from mice treated with 3000 mg/kg aqueous extract showing mild tubular degeneration with preservation of the glomerular architecture ($\times 40$).

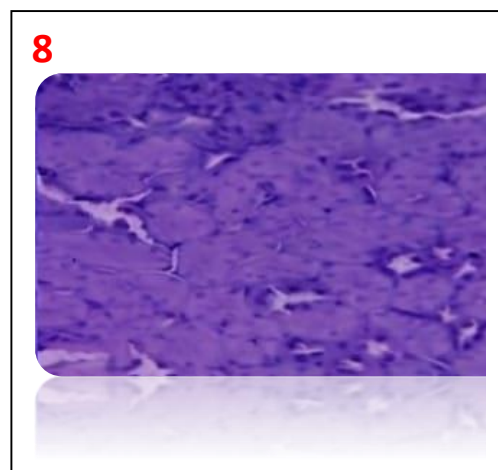


Plate 8. Photomicrograph of the kidney section from mice treated with 5000 mg/kg aqueous extract showing mild tubular vacuolation with preserved renal corpuscles and no evidence of severe tissue damage ($\times 40$).



Plate 9. Photomicrograph of the liver section from mice treated with 1000 mg/kg methanolic extract showing normal hepatic architecture with intact hepatocytes and preserved sinusoidal spaces (x40).

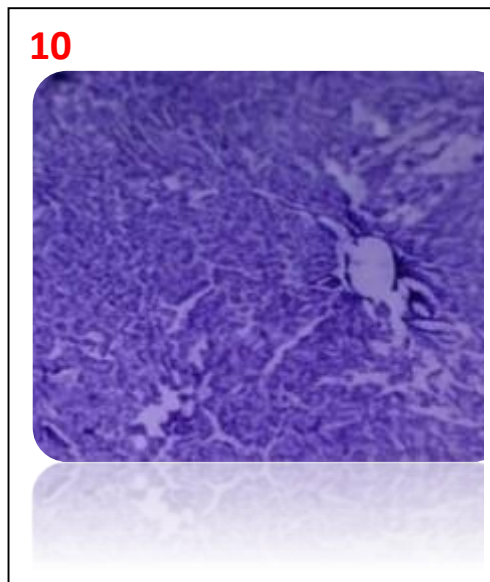


Plate 10. Photomicrograph of the liver section from mice treated with 3000 mg/kg methanolic extract showing mild hepatocellular changes with slight sinusoidal congestion while maintaining overall hepatic architecture (x40).

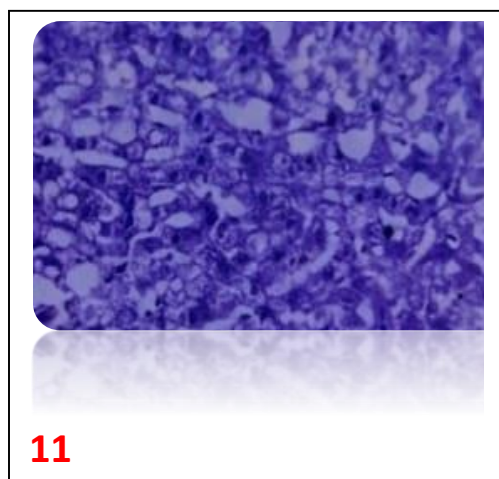


Plate 11. Photomicrograph of the liver section from mice treated with 5000 mg/kg methanolic extract showing well-preserved hepatic tissue with only minimal histological alterations, indicating no marked hepatotoxic effect (x40).

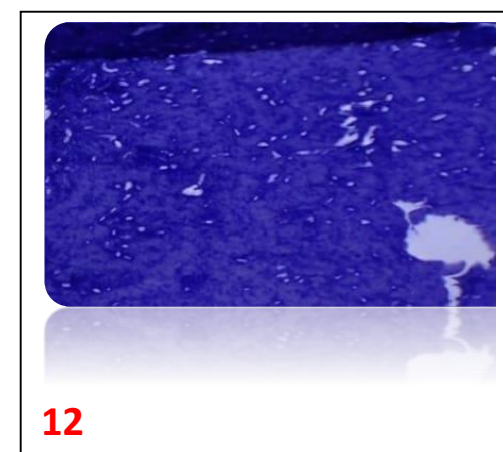


Plate 12. Photomicrograph of the kidney section from mice treated with 1000 mg/kg methanolic extract showing mild mesenchymal changes with preserved glomeruli and renal tubules (x40).

DISCUSSION

The qualitative and quantitative screening demonstrated that *L. microcarpa* leaves contain diverse secondary metabolites, with extraction yields strongly influenced by solvent polarity. The presence of alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids aligns with findings by Sombié *et al.* (2011) and Ouedraogo *et al.* (2022), who reported rich polyphenolic profiles in West African *L.*

microcarpa leaves and bark. The higher yield of total phenolics and terpenoids in methanol parallels observations by Nitiéma *et al.* (2023), who demonstrated that alcoholic solvents show greater efficiency in extracting moderately polar phenolic compounds and lipophilic triterpenoids. The higher concentration of flavonoids and saponins in the aqueous extract agrees with Umar and Ayuba (2022), who observed that polar water molecules favor the

recovery of glycosylated flavonoids and hydrophilic saponin structures.

However, a study conducted by Ndjoubi *et al.* (2019) in Central Africa reported lower total phenolic yields and an absence of detectable alkaloids in *L. microcarpa* leaf samples, which does not agree with the present findings. Similarly, Kaboré *et al.* (2017) observed higher saponin yields in alcoholic fractions than in aqueous media, which also disagrees with the present study. These discrepancies may be attributed to geographical variation, soil nutrient composition, microclimatic conditions, seasonal harvesting differences, and extraction parameters such as maceration duration and temperature (Mangla *et al.*, 2021).

The absence of mortality or severe clinical signs up to the limit dose of 5000 mg/kg b.w. establishes the median oral lethal dose (LD₅₀) as greater than 5000 mg/kg b.w., classifying both extracts as practically non-toxic under OECD guidelines. This high safety margin agrees with acute toxicity studies by Owusu and Antwi-Adjei (2017), Kinda *et al.* (2021), and Ouattara *et al.* (2022), which reported no acute mortality in rodents administered *L. microcarpa* leaf or stem bark preparations at doses exceeding 2000 to 5000 mg/kg b.w. These findings also parallel Shehu *et al.* (2024), who reported LD₅₀ > 5000 mg/kg b.w. for *Acacia nilotica* leaf extracts in Swiss albino mice.

However, a study conducted by Ogwal *et al.* (2020) on *Spondias mombin*, a related Anacardiaceae species, recorded mild toxic reactions and lower LD₅₀ values, which does not agree with the present findings. Similarly, Pale *et al.* (2021) observed acute toxic signs when using *Lannea acida* stem bark extracts at lower thresholds, which also disagrees with the present study. These divergences may be attributed to plant part specificity and chemical composition, as stem barks tend to accumulate higher concentrations of cytotoxic condensed tannins and proanthocyanidins, whereas leaves contain higher levels of antioxidant flavonoids such as vitexin, epicatechin, and myricetin derivatives that protect cell membranes from radical damage (Mangla *et al.*, 2021; Ouedraogo *et al.*, 2022). The 21-day repeated-dose study showed no significant differences ($p > 0.05$) in body weight gain, relative organ weights, serum hepatic enzymes (ALT, AST, ALP), renal filtration markers (urea, creatinine), or serum electrolytes (Na⁺, K⁺, Cl⁻) between treated mice and controls. These findings were supported by histopathological analysis showing preserved liver and kidney architecture. These sub-acute biochemical and histological findings agree with Belemnaba *et al.* (2020) and Ilboudo *et al.* (2023), who demonstrated

that sub-chronic oral administration of standardized *L. microcarpa* bark formulations (LAMIC) caused no significant hepato-renal toxicity or histopathological damage in rodents. Similarly, Abebe (2023) and Shehu *et al.* (2024) observed that polyphenol-rich plant extracts did not impair liver or kidney biomarkers in rodent safety models.

However, a study conducted by Obidike *et al.* (2025) reported that repeated oral administration of *Khaya anthotheca* stem bark extract at doses above 250 mg/kg b.w. induced hepatic enzyme elevation, focal hepatocellular necrosis, and renal tubular damage, which does not agree with the present findings. Additionally, Easmin *et al.* (2015) recorded nephrotoxicity in mice exposed to fruit extracts containing cytotoxic triterpenes, which also disagrees with the present study. The safety of *L. microcarpa* leaf extracts observed in this study may be attributed to the absence of cytotoxic limonoids or reactive alkaloidal toxins, as well as the presence of hepatoprotective polyphenols that support endogenous antioxidant systems such as superoxide dismutase and catalase, preventing cellular injury during 21-day metabolism (Ilboudo *et al.*, 2023; Ouedraogo *et al.*, 2022). The minor sinusoidal dilation and localized tubular swelling observed at 5000 mg/kg b.w. represent adaptive physiological responses to high metabolic loads rather than pathological necrosis, supporting the overall safety of the extracts.

CONCLUSION

African grape (*Lannea microcarpa*) leaves contain diverse bioactive secondary metabolites, with extraction yield depending on solvent polarity. Methanol enhances the recovery of phenolics, terpenoids, and steroids, whereas water extracts higher amounts of flavonoids and saponins. Both extracts were well tolerated by mice, with an oral median lethal dose (LD₅₀) greater than 5000 mg/kg b.w. Repeated daily administration for 21 days caused no significant hepatotoxicity or nephrotoxicity, as confirmed by serum biochemical markers and organ histopathology. These findings support the traditional ethnoveterinary use of *L. microcarpa* leaves and establish a solid safety baseline for future evaluations of their therapeutic efficacy.

AUTHOR CONTRIBUTIONS

Umar Abdullahi conceived and designed the study, performed the laboratory experiments, analyzed and interpreted the data, and drafted the manuscript. Dr. James Bmnshima Orpin supervised the research, provided technical guidance, and critically revised the

manuscript for important intellectual content. Mr. Abdulhadi Bawa Jibia contributed to the experimental work, data collection, and statistical analysis. All authors read and approved the final version of the manuscript before submission.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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ETHICAL APPROVAL

All animal procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Ibrahim Badamasi Babangida University (IBBUL), Lapai, and the Organization for Economic Co-operation and Development (OECD) guidelines. Ethical approval was obtained from the Institutional Animal Care and Use Committee prior to the commencement of the study.

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