



Renal Histopathological Effects of *Afzelia africana* n-Hexane Seed Oil Extract Following Sub-Chronic Oral Exposure in Wistar Rats

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DOI: <https://dx.doi.org/10.4314/aja.v15i1.7>

Conflict of interest: None

Funding: None

Abstract

Background: *Afzelia Africana*, commonly known as African Mahogany, is widely utilised across sub-Saharan Africa for nutritional and ethnomedicinal purposes. While the seed might be safe in traditional dietary amounts, the development of therapeutic concentrated lipophilic extracts introduces new toxicological variables. The renal system's high metabolic demand for processing concentrated lipids necessitates a rigorous safety evaluation. This study evaluated the renal histopathological effects of repeated oral administration of an n-hexane extract of *A. africana* seed oil in Wistar rats.

Methods: Twenty adult male Wistar rats were randomly assigned into four groups (n = 5 per group): control and three treatment groups receiving 1000, 1250, and 1500 mg/kg body weight of the extract daily for 28 days. Renal toxicity was assessed using body weight measurements and a semi-quantitative histopathological scoring system applied to hematoxylin and eosin-stained kidney sections.

Results: Final body weights were significantly reduced in rats treated with 1000



mg/kg and 1250 mg/kg compared with controls ($p < 0.05$), while rats receiving 1500 mg/kg showed no significant difference. Histological examination revealed mild tubular fatty change and interstitial inflammation at 1000 mg/kg, preserved renal architecture at 1250 mg/kg, and early nephrotoxic changes including tubular degeneration, vascular congestion, and inflammatory infiltration at 1500 mg/kg. The observed pattern suggests a non-monotonic dose-response relationship.

Conclusion: Sub-chronic exposure to *A. africana* n-hexane seed oil extract induces dose-dependent renal histopathological changes with a non-monotonic profile. While 1250 mg/kg appeared to be a tolerable dose under the conditions of this study, early nephrotoxic alterations were evident at 1500 mg/kg. These findings indicate a narrow renal safety margin for lipophilic extracts of *A. africana* and underscore the need for caution in the use of non-polar solvent-derived botanical preparations.

Keywords: *Afzelia africana*, Renal histopathology, Sub-chronic toxicity, Seed oil extract

Introduction

The mammalian kidney is very susceptible to injury by extraneous compounds due to the significant volume of cardiac blood supply that passes through the organ and the filtering that leads to the concentrating of these wastes in the tubules (1). In sub-Saharan Africa, traditional medicine remains the mainstay of primary health care, but the transition from traditional brews to packaged medicinal plants is raising fears about the reliability of the dosages and the toxic potential of the solvents (2,3). The complex vascular structure of the renal tissue is very susceptible to phytochemicals that activate oxidative stress and inflammation (4). The histological changes induced by these agents, including necrosis of the tubules and thickening of the basement membrane of the glomeruli, may be seen even if blood tests, including blood urea and creatinine levels, are normal (5). The histopathological changes are therefore critical in the early detection of kidney



injuries (6,7).

Afzelia africana is a common plant used in West and Central African traditional medicine, thanks to its antimicrobial and anti-inflammatory properties (8). The seeds are an essential source of protein in nutrition which have been reported to exhibit dose-dependent biological effects, including potential nephrotoxicity (9). Most of the studies performed so far were done with aqueous or alcoholic extracts, but results cannot be directly transposed to a lipophilic fraction obtained with non-polar solvents (10). The extraction methodology of the plant influences its toxicological profile: n-hexane, for example, extracts hydrophobic compounds like sterols and fatty acids, which could present their own biological and toxic action (11,12).

Lipophilic phytochemicals are believed to exert toxicity partly through oxidative stress-related pathways. Currently, oxidative stress is viewed not just as a side effect of tissue injury but increasingly recognized as a constituent of redox signaling and regulation (13,14). Since *A. africana* seeds contain substantial quantities of lipids, ranging from

approximately 37%, safety data on the sub-chronic toxicity of its n-hexane oil fraction are conspicuous by their absence (15,16). Additionally, variations in hemato-chemical parameters often represent markers of systemic adaptation or stress responses consequent to exposure to botanicals; nonetheless, such responses are rarely correlated with changes in renal histomorphology (17).

Despite the widespread ethnomedicinal use of *Afzelia africana*, comprehensive data on sub-chronic renal histopathological effects of n-hexane extract, particularly following repeated oral exposure to its lipophilic fractions, are limited. Existing studies have largely focused on acute toxicity, indicating no mortality in Wistar rats at doses up to 5000 mg/kg (15), as well as preliminary evaluations of its chemical composition (16). However, systematic histopathological investigations necessary to elucidate dose-dependent organ-specific toxicity are lacking. Establishing the threshold at which nutritionally valuable seeds may exert nephrotoxic effects is critical for public health risk assessment (18). Against this background, the present study aimed to



investigate dose-dependent renal histopathological alterations induced by an n-hexane seed oil extract of *Afzelia*

africana in Wistar rats using standardized extraction and scoring methods (19).

Materials and Methods

Study Design

The study design used was based on a controlled in vivo experimental animal study designed to assess dose-dependent renal histopathological changes induced by *Afzelia africana* n-hexane seed oil extract in Wistar rats. All procedures were conducted in accordance with specific toxicological and animal welfare guidelines approved for the study with measures taken to enhance reproducibility, minimize bias and ensure reproducibility, minimize bias, and ensure ethical handling of the animals

Preparation of n-Hexane Seed Oil Extract

To obtain the lipophilic constituents of interest for the study, a standard procedure with n-hexane extraction was employed. Dried samples were dehulled mechanically to eliminate the non-volatile outer shell. Subsequent

mechanical grinding was performed to produce a fine powder. Soxhlet extraction was performed on the powdered sample with analytical grade n-hexane (Merck, Germany) as the extraction solvent. These operations were performed until the solvent collection line changed to colorless, signifying the efficient extraction of the targeted compounds. Extraction was terminated, and the removal of the n-hexane was performed by a rotary evaporator (Buchi-R-210, Switzerland) at temperatures below 40°C as the vacuum pressure was reduced (19).

Experimental Animals

A total of twenty adult male Wistar rats weighing between 220–260 g was employed for this study. The rats were acclimatized for two weeks under controlled laboratory conditions of 12-hour light/dark cycles, 22 ± 2 °C, and 50–60% humidity. The animals were given free access to standard rodent chow and water. Housing, handling,



and euthanasia were performed in strict compliance with ethical treatment concerns in the care and use of laboratory animals. The preparation of experimental animal models was in compliance with the principles of animal research as recommended by the Declaration of the American Physiological Society's Guiding Principles in the Care and Use of Animals, 2002. The study was conducted in compliance with the ARRIVE 2.0 guidelines for reporting animal research. This research was approved by the Animal Research Ethics Committee, Lagos State University of Medicine, Ikeja, Lagos State (Ref No: AREC/2023/001).

Experimental Design and Dosing

To evaluate the dose-dependent renal histopathological effects, the Wistar rats were randomised into four experimental groups, each comprising five animals (n=5 per group), after the acclimatisation period. The experimental groups were constituted as follows:

Group I (Control): Received an equivalent volume of distilled water only.

Group II: Administered *Afzelia africana* n-hexane seed oil extract at 1000 mg/kg body weight/day (low dose).

Group III: Administered *Afzelia africana* n-hexane seed oil extract at 1250 mg/kg body weight/day (medium dose).

Group IV: Administered *Afzelia africana* n-hexane seed oil extract at 1500 mg/kg body weight/day (high dose).

The dose range chosen is based on previous acute toxicity studies, in which *A. africana* seed extracts have shown an oral LD₅₀ of above 5000 mg/kg, indicating a high safety margin in acute settings [15,16]. On that basis, the starting doses 1000 mg/kg was selected as a high exploratory dose based on previous acute toxicity reports indicating an LD₅₀ > 5000 mg/kg for the safety evaluation of non-toxic botanical substances (20). To accurately delineate the boundary, dose increments of 250 mg/kg were selected to allow finer resolution of dose-



dependent renal histopathological changes (12).

Tissue Collection and Histological Processing

At the end of the 28 days, the test animals were fasted overnight and euthanized according to proper anesthesia, as recommended in the guidelines toward caring for animals used in a lab. An incision along the midline was used to open the abdominal cavity, and both kidneys were removed and cleaned by gently rinsing them with cold normal saline solution to remove blood, then patted dry.

Representative tissue samples of the kidneys were immediately fixed in 10 percent neutral buffered formalin, where they were preserved for at least 24 to 48 hours. Thereafter, the tissue samples were subjected to the routine process, which included dehydration, clearing, and incorporation of the tissues in wax, all as described in the histology process. Kidney sections embedded in paraffin were sectioned at 4-5 μm thickness using a rotary microtome. These sections were

collected and placed on clean glass slides. The sections were then subjected to the process of deparaffinization, hydration, and staining by hematoxylin and eosin stain using routine procedures. The sections were then examined under a microscope to observe the histoarchitecture of the kidneys. Multiple tissue sections were prepared, and representative sections were randomly selected for analysis to minimize selection bias and ensure reproducibility of histopathological observations.

Histopathological Evaluation and Scoring

A veterinary pathologist, unaware of the random assignments, evaluated the H&E-stained sections of the kidneys under a light microscope. In the sections of the kidneys of the rat, five to ten non-overlapping fields under the microscope at $\times 400$ magnification were chosen for an unbiased view of the renal cortex and medulla. Lesions were semi-quantitatively scored on a four-point ordinal scale (21): 0 (Absent); 1 (Mild, affecting <25% of the field); 2 (Moderate, affecting 25-50% of the



field); and 3 (Severe, affecting >50% of the field). The specific morphological domains assessed included:

Tubular-epithelial

degeneration/vacuolation: indicators of acute cellular stress and cytoplasmic injury.

Interstitial inflammation: presence of mononuclear leucocytic infiltration reflecting a localized immune response.

Vascular hemorrhage/congestion: evidence of impaired microcirculation or breakdown of vascular integrity.

Glomerular-structural alteration: encompassing architectural distortions such as capillary tuft contraction or Bowman's space changes.

The dominant lesion score across the examined microscopic fields was

recorded for each animal. These individual scores were then used to calculate the group median and interquartile range (IQR) for statistical comparison.

Statistical Analysis

Continuous variables for body weight were expressed as mean $\pm$ standard deviation (SD) and analysed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Data distribution was assessed prior to inferential analysis. Ordinal histopathological scores were scored. Data distribution was assessed for normality prior to analysis. Statistical significance for inferential tests was set at $p < 0.05$.



Results

Physical Parameters and Body Weight Observations

Final body weight differed significantly among groups following 28-day repeated dose of *Afzelia africana* n-hexane seed oil (one-way ANOVA, $F(3,16) = 120.91$, $p < 0.001$). Rats treated with 1000

mg/kg and 1250 mg/kg exhibited significantly lower final body weights compared with the control group (Tukey's HSD, $p < 0.05$). In contrast, animals receiving 1500 mg/kg showed a final body weight that did not differ significantly from controls ($p > 0.05$)

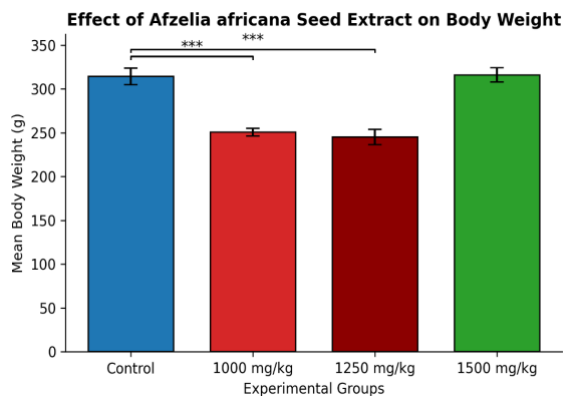
Table 1: Final Body Weights of Wistar Rats Following Exposure to *Afzelia africana* Seed Extract

Experimental Group	n	Dose (mg/kg)	Final Body Weight (g)
Group I (Control)	5	Vehicle	314.2 ± 9.4 ^a
Group II (Low Dose)	5	1000	250.6 ± 4.2 ^b
Group III (Medium Dose)	5	1250	245.0 ± 8.8 ^b
Group IV (High Dose)	5	1500	316.0 ± 8.2 ^a

Legend: Effect of sub-chronic oral administration of *Afzelia africana* n-hexane seed oil on final body weight of Wistar rats. Values are expressed as mean ± SD (n = 5). Groups sharing the same superscript letter are not significantly different. Different letters indicate significant differences (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$).



Figure 1: Effect of *Afzelia africana* seed extract on body weight of Wistar rats following 28 days of oral administration.



Legend: Data are presented as mean $\pm$ SD (n = 5 per group). Rats administered medium (1250 mg/kg) and low (1000 mg/kg) doses showed reduced body weight compared with the control and high-dose (1500 mg/kg) groups. Statistical analysis was performed using one-way ANOVA, with significance set at $p < 0.05$.

Renal Histopathology

Histological evaluation of renal cortical sections demonstrated a progressive, dose-dependent pattern of tubulointerstitial injury. Lesions were assessed semi-quantitatively using an ordinal grading system (0–3) based on estimated proportion of affected tissue across multiple microscopic fields per section. Representative photomicrographs from each group are provided in Figure 2.

Control group (score 0): Renal cortex

exhibited intact glomeruli, uniform tubular epithelium, no vacuolation, necrosis, or interstitial inflammation, and normal vasculature. Low-dose 1000mg/kg, group (score 1, <25% involvement): Mild focal cytoplasmic vacuolation and hydropic swelling of tubular epithelial cells, occasional mononuclear interstitial infiltrates, preserved tubular basement membranes and glomeruli. Intermediate-dose group, 1250mg/kg, (score 2, 25–50% involvement): Moderate tubular epithelial degeneration with multifocal interstitial inflammation, luminal irregularity, limited early necrotic foci. High-dose group, 1500 mg/kg (score 3, >50% involvement): Extensive tubular degeneration and necrosis, diffuse interstitial inflammation, vascular congestion, focal hemorrhage, and mild to moderate glomerular distortion.

Comparative Dose-Response Summary of Renal Structural Findings

Histological assessment was performed on hematoxylin and eosin-stained kidney sections using light microscopy. Parameters evaluated included tubular epithelial degeneration, interstitial inflammation, vascular congestion or



hemorrhage, and fatty change. Scores observed across animals within each represent group-level values derived experimental group. from consistent microscopic findings

Table 2: Incidence and semi-quantitative severity of renal histopathological lesions following 28-day oral exposure to *Afzelia africana* seed oil.

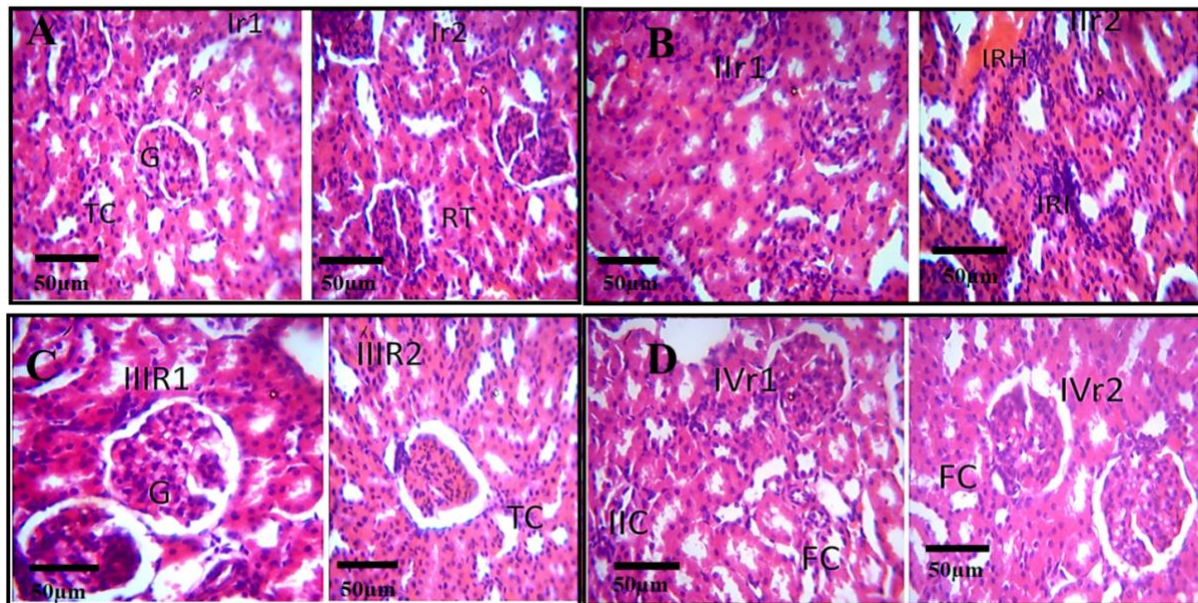
Histopathological Feature	Control (n=5)	1000 mg/kg (n=5)	1250 mg/kg (n=5)	1500 mg/kg (n=5)
Tubular Degeneration	0/5 (0)a	0/5 (0)a	0/5 (0)a	4/5 (1)b*
Interstitial Inflammation	0/5 (0)a	3/5 (1)b**	0/5 (0)a	5/5 (1)c**
Vascular Congestion	0/5 (0)a	0/5 (0)a	0/5 (0)a	3/5 (1)b
Intratubular Fatty Change	0/5 (0)a	2/5 (1)b	0/5 (0)a	0/5 (0)a

Legend: Values represented as number affected/total with median lesion scores in parentheses. Composite Score = median (IQR) of the sum of all individual lesion scores. Scoring Scale: 0 = Normal architecture, 1 = Mild (<25% affected), 2 = Moderate (25% to 50% affected), 3 = Severe (>50% affected with necrosis). Lesion scores were analyzed using the Kruskal-Wallis H-test followed by Dunn's multiple comparison test for all four (4) groups. The composite pathology scores were analyzed with the Kruskal-Wallis H-test: H (3) =17.82, p=0.0005 Letters (a, b, c) within a row represent statistical differences between the groups (p < 0.05). Asterisks refer to differences between the control group and the other groups (* p < 0.05 and ** p < 0.01)



Histological Analysis of Renal Tissues

Figure 2: Representative Photomicrographs of Renal Cortex in Wistar Rats Following Oral Administration of *Afzelia africana* n-Hexane Seed Oil Extract



Legend: (A) Control group showing normal renal architecture with well-defined glomeruli (G), renal tubules (RT), and tubular cells (TC). (B) High dose group (1500 mg/kg) showing mild interstitial inflammatory cell infiltration and focal intrarenal hemorrhage (IRH), with preserved glomerular structure and identifiable tubular cells. (C) Medium dose group (1250 mg/kg) showing intact renal architecture with clearly outlined glomeruli (G) and active tubular cells (TC), without obvious structural distortion. (D) Low dose group (1000 mg/kg) showing mild fatty change (FC) within tubular epithelium and minimal inflammatory cell infiltration (IIC), with preserved glomeruli and tubular outlines. Hematoxylin and eosin (H&E) stain; magnification $\times 400$.



Discussion

Histopathological examination of renal tissue constitutes the primary outcome of this investigation. Assessment across the three dose levels revealed a non-linear dose-response relationship in renal tissue. At 1000 mg/kg, mild intratubular fatty change was observed in 2 of 5 animals, accompanied by mild interstitial inflammatory cell infiltration

in 3 of 5 animals (Table 2, Figure 2D). The presence of fatty change in tubular epithelium at this dose is consistent with findings reported in studies involving lipophilic plant extracts, where tubular epithelial cells represent early targets of metabolic perturbation due to their high mitochondrial activity and role in xenobiotic handling (22).

Table 3: Summary of the histopathology of the kidneys across the experimental groups following graded oral administration of *Afzelia africana* n-hexane seed oil extract.

Dose Category	Dose (mg/kg)	Dominant Histological Findings	Toxicological Interpretation
Control	0	Normal glomeruli and tubules; no inflammation	Physiological baseline
Low Dose	1000	Mild fatty change; mild inflammatory cell infiltration	Early adaptive or metabolic stress response
Medium Dose	1250	Preserved renal architecture	Apparent safety threshold
High Dose	1500	Mild hemorrhage and interstitial inflammation	Early nephrotoxic injury

Legend: An overview of the salient microscopic features of each dose group following the examination of hematoxylin and eosin-stained sections using a light microscope. The examination was carried out by comparing the changes of the kidneys across each dose group.



Furthermore, renal tubular cells are particularly vulnerable to lipid accumulation and metabolic stress due to their dependence on oxidative metabolism, making them susceptible to early reversible injury prior to overt necrosis (5). At 1250 mg/kg, renal architecture was entirely preserved across all five animals, with no observable tubular degeneration, interstitial inflammation, vascular congestion, or fatty change (Table 2, Figure 2C). This histological integrity at the intermediate dose distinguishes it from both the lower and higher dose groups, and is consistent with a narrow adaptive window within which renal homeostatic mechanisms may buffer xenobiotic stress. Similar transient adaptive responses have been described in toxicological systems where moderate exposure levels activate cytoprotective pathways, including antioxidant defenses and stress-response signaling (13).

The concurrent reduction in body weight at this dose indicates that systemic physiological stress may occur independently of overt renal morphological changes, a phenomenon

previously reported in sub-toxic exposure models where functional or metabolic disturbances precede structural injury (6). Significant renal injury was observed at 1500 mg/kg, where tubular degeneration, interstitial inflammation, and vascular congestion (Table 2, Figure 2B). These findings indicate a dose-associated nephrotoxic response characterised by tubular epithelial injury, inflammatory infiltration, and microvascular involvement. The predominance of tubular injury is consistent with established nephrotoxicity models, in which proximal tubular cells are primary targets due to their role in reabsorption and concentration of xenobiotics (22).

The observed mononuclear leukocyte infiltration likely represents a secondary inflammatory response to tissue injury, rather than a primary immunogenic effect, as described in toxicant-induced renal damage [5]. Phytochemical constituents of *Afzelia africana*, including alkaloids and saponins, have been reported to exert membrane-disruptive and cytotoxic effects on epithelial and endothelial cells (5,21); however, their specific contribution to the present



findings remains hypothetical and requires direct phytochemical histopathological correlation (23). Similarly, oxidative stress has been widely implicated in tissue injury through mechanisms involving reactive oxygen species and lipid peroxidation, which are common denominators in many forms of toxicant and ischemia-reperfusion damage (13), but the absence of oxidative stress biomarkers in this study precludes mechanistic attribution.

Taken together, the renal histopathological data indicate a non-monotonic dose-response pattern, with structural injury evident at both low and high doses, and relative preservation at the intermediate dose. This pattern deviates from classical linear toxicological responses and is consistent with biphasic dose-response dynamics, where low and high exposures may produce qualitatively different biological effects (24). Such responses may reflect a dynamic interplay between cellular stress, adaptive signaling, and toxic overload. Within a limited exposure range, compensatory mechanisms including antioxidant activation and

cellular repair pathways may maintain structural integrity, whereas these mechanisms become overwhelmed at higher concentrations. Similar narrow margins of renal tolerance have been reported for lipophilic phytochemical-rich extracts, underscoring the importance of dose precision in toxicological literature (6).

With respect to body weight, a secondary systemic endpoint, Rats administered 1000 mg/kg and 1250 mg/kg demonstrated significantly reduced body weights relative to controls, while the 1500 mg/kg group showed no significant difference (Table 1, Figure 1). This pattern further reflects a non-linear response, with maximal weight suppression at intermediate exposure. One possible explanation lies in physiological adaptation, where higher levels of stress may activate compensatory endocrine and metabolic responses, including glucocorticoid signaling and metabolic reprogramming, which transiently preserve body weight despite underlying tissue injury (25, 13).

The present findings challenge the assumption that ethnobotanical preparations are inherently safe



irrespective of dose and extraction method. The observed non-linear responses across both structural and systemic endpoints underscore the complexity of toxicological evaluation of lipophilic plant fractions and reinforce

Conclusion

This study establishes a histologically defined tolerability threshold for the n-hexane seed oil extract of *Afzelia africana* under sub-chronic exposure conditions. The findings indicate a dose-associated pattern of tubulointerstitial injury, characterized by progressive tubular epithelial damage and increasing inflammatory involvement at higher exposure levels. The dose-response pattern observed indicates that 1250 mg/kg represents the highest dose without detectable adverse renal effects, while 1000 mg/kg elicited mild adaptive changes and 1500 mg/kg induced early nephrotoxic injury. These findings highlight the narrow safety margin associated with lipophilic botanical extracts and challenge the assumption that traditionally consumed plant products are inherently safe when processed using non-polar organic solvents.

the need for dose standardization and multi-endpoint assessment in future studies.

Study Limitations

First, the absence of parallel phytochemical characterization and antioxidant biomarker assessment limits the ability to establish definitive mechanistic links between the observed histopathological alterations and specific bioactive constituents or oxidative stress pathways.

Second, renal function biomarkers such as serum creatinine and blood urea nitrogen (BUN) were not evaluated. As a result, the functional significance of the observed structural alterations cannot be fully determined, and it remains unclear whether the histological changes correspond to measurable impairment in renal function.

Furthermore, food intake was not monitored during the experimental period. Consequently, the observed changes in body weight cannot be definitively attributed to treatment-



related metabolic effects, as reduced caloric intake or altered palatability may have contributed to the observed differences. Likewise, the relatively small sample size ($n = 5$ per group) may limit statistical power and increase susceptibility to inter-individual variability, thereby affecting the generalizability of the findings.

Acknowledgements

The authors acknowledge the technical assistance of the staff of the Department of Anatomy, EKo University of Medicine and Health Sciences, Nigeria, for their support with animal handling and

laboratory procedures. We also appreciate the ethical oversight provided by the Animal Research Ethics Committee, Lagos State University of Medicine. The authors are grateful to colleagues who provided critical feedback during the preparation of the manuscript.

Data availability statement

The datasets generated during this study are available from the corresponding author upon reasonable request.



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