ORIGINAL ARTICLE

# Acute and Sub- acute Toxicity of Methanol Root Extract of *Azanza Garckeana (Malvaceae*) in Albino Rats

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## ABSTRACT

**Background:** Azanza garckeana (A. garckeana) is a widely used traditional medicinal plant known for treating various human ailments. This study aimed to evaluate the acute and subacute toxicity of its methanol root extract in albino rats.

**Methods:** A total of 24 rats were divided into four groups, with 6 rats in each group. Three groups received oral doses of 150, 300, and 600 mg/kg of the extract daily orally for 28 days, while the control group received saline daily orally for the same period. Throughout the study, clinical signs of toxidromes such as changes in skin and fur, eyes, mucous membranes, mydriasis, miosis, tremors, convulsions, salivation, diarrhoea, sedation, coma and mortality were monitored. On day 29, blood samples were collected for haematological and biochemical analysis, followed by euthanization and histopathological examination of the heart, liver, and kidneys.

**Results:** The results revealed a significant increase to "22 $x10^3/\mu L$" ($P<0.001$) in white blood cell (WBC) counts in all treatment groups compared to the control and the increase was dose dependent. Additionally, significant elevations by 67% ($P=0.01$) in the two liver enzymes AST and ALT were observed in the treatment groups. Histopathological evaluation showed notable kidney damage in the 600 mg/kg group, including glomerular shrinkage, focal segmental glomerulosclerosis (FSGS), and focal tubular necrosis. Liver tissues from the 300 mg/kg and 600 mg/kg groups exhibited chronic inflammation and fibrosis.

**Conclusion:** Repeated administration of A. garckeana methanol root extract induced subacute toxicity in albino rats, particularly affecting the liver and kidneys. The severity of histopathological changes suggested a dose-related pattern of organ toxicity. Caution is advised in the prolonged medicinal use of this plant by traditional healers.

## INTRODUCTION

Nature has long served as a rich source of therapeutic agents, with plant-based products continuing to play a pivotal role in primary health care for approximately 80–85% of the global population.[1] The increasing public and professional acceptance of medicinal plants is attributed to advances in understanding their mechanisms of

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action and their positive influence on health and quality of life.[2] While medicinal plants are widely regarded as safe and are used to manage various diseases, some can pose significant risks if consumed in excessive doses or in combination with conventional drugs.[3] Therefore, assessing the safety and toxicological profiles of medicinal plants intended for human or animal use is essential.

*Azanza garckeana* (*A. garckeana*), a multipurpose plant native to tropical Africa, has been extensively used in traditional medicine to manage numerous health conditions. The bark, fruits, leaves, roots, and stems of the plant are documented to possess diverse therapeutic properties.[4] Literature from nine African countries representing 64.3% of the plant's native range records at least 22 traditional medicinal uses of *A. garckeana*.[4] The plant's roots and other parts have been used to treat ailments such as chest pain, cough, menstrual irregularities, and sexually transmitted diseases, including gonorrhoea and syphilis.[5, 6, 7, 8] Additionally, *A. garckeana* has been traditionally employed for its uterotonic properties.[9] Of particular interest is the use of the root either alone or in combination with stem bark to treat infections and reproductive health issues, which suggests the presence of bioactive compounds with systemic effects. Despite the widespread traditional use of *A. garckeana*, especially its roots, comprehensive toxicological studies remain limited. A previous study demonstrated that repeated administration of methanol extracts of the plant's pulp affected biochemical markers such as urea, uric acid, creatinine, and electrolytes, particularly at higher doses. However, that study did not investigate haematological, histopathological, or clinical signs of toxicity, nor did it evaluate the root extract specifically.[11]

Given the frequent traditional use of the plant extract and its suspected systemic bioactivity, it is critical to assess the safety of the methanol root extract of *A. garckeana*. Although *A. garckeana* is traditionally prepared and consumed as an aqueous (water-based) extract, methanol is widely used in scientific research as an extraction solvent because of its ability to extract a broader range of phytochemicals, including both polar and moderately non-polar compounds. Methanol extracts often contain higher concentrations of bioactive compounds, allowing for a more comprehensive toxicological evaluation. Investigating the methanol root extract therefore provides insight into the full spectrum of potential bioactive constituents and helps identify any toxic compounds that might not be captured through traditional water extraction methods.[9] The present study was therefore designed to evaluate the potential toxic effects of this extract in albino rats, with a focus on a broad range of biochemical, haematological, and histopathological parameters.

## MATERIALS AND METHODS

### Research Design

The study was an experimental randomized controlled study, which was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 407.

### Plant Collection

The fresh roots of *A. garckeana* were collected from Chongwe (rural) Lusaka, Zambia on 9th december,2021, with the following google location (-15.2643320, 28.5107680). The plant was taken for taxonomic identification and authentication at the herbarium, School of Natural Sciences, University of Zambia and the plant was identified with accession number 22209 (UZL).

### Plant Material Extraction

The process of extraction was done according to the procedure by Chanda *et al.*, using cold maceration.[9] The roots were washed to remove debris and then air dried in the shade at room temperature for 14 days. They were then ground to a uniform powder using a mortar and pestle then using a blender (1.75 litres Satin Russell Hobbs Blender, India). The powder (260g) was macerated in 99.5% methanol (2000mls) at room temperature (22 - 26 ℃) and was allowed to stand for 72 hours with periodic agitation which

maximized extraction of the chemical constituents from the plant.

The extract was then filtered through the Whatman no. 1 filter paper (Merck, Germany), and the filtrate was evaporated to a paste with the aid of a water bath at 40 ℃. The residue was weighed, and the percentage yield was determined. The residue was then stored in air and water-proof plastic containers and then kept at 4°C in the refrigerator until when it was required for experimental use.

The percentage yield was determined by the following calculation:

Percentage Yield = Weight of sample extract obtained (g) X 100 = 28.5g/260gx100 =11%
Weight of the powdered sample used (g)

**Experimental Animals**

Healthy male and female albino rats aged 8 weeks, weighing between 110-170g were randomly chosen and obtained from the Department of Biology at University of Zambia. The rats were habituated in metal cages for 2 weeks at room temperature 22°C (±3°C) with adequate ventilation and a 12-hour light/dark cycle maintained.

The sample size of 6 animals per group (24 total) was determined based on a previous toxicological study conducted by Yusuf et al. (2020), which assessed the effects of methanol extracts of *Azanza garckeana* in a comparable rat model using similar dosing schedules. This approach aligns with standard practice in preclinical toxicological assessments and is consistent with OECD guideline 407 for repeated dose 28-day oral toxicity study in rodents.

**Experimental Design**

The route of administration was by oral gavage in accordance with the main route of intake of *A. garckeana* decoction by humans for medicinal purposes. The six animals in each group received daily single dosing treatments for 28 days according to the information given in Table 1.

**Table 1: Animal dosing schedule during the study**

| Group | Drug | Dosage | Duration |
|---|---|---|---|
| Group 1 | Normal Saline | 2mls/kg | 28 days |
| Group 2 | A. garckeana extract | 150mg/kg | 28 days |
| Group 3 | A. garckeana extract | 300mg/kg | 28 days |
| Group 4 | A. garckeana extract | 600mg/kg | 28 days |

The dosages were determined from the previous study by Yusuf *et al*. on the same plant species.[11] Following daily single dosing treatments, the rats had free access to food and water for the rest of the experimental period.

**Clinical observations**

The animals were observed daily over a 28-day period following administration of the methanol root extract. This monitoring was performed on individual animals at least once within the first 30 minutes post extract administration followed by 4-hour intervals over 24 hours. Special attention was given during the first 4 hours for the acute period (14 days) and daily thereafter. Clinical signs of toxidromes were observed using the Toxicological Severity Scoring Index (TSSI) and changes in skin and fur, eyes, mucous membranes, mydriasis, miosis, tremors, convulsions, salivation, diarrhoea, lethargy, sedation, coma and mortality were observed especially in the acute period (within 14 days).

Time and day of onset was recorded. Body weights were measured immediately before starting the dosing, weekly thereafter and at the end of the treatment period.

**Collection of samples**

On the 29$^{th}$ day, 3mls of blood was collected by cardiac puncture from each animal of which 1ml was put into heparinized ethylene diamine tetra acetate (EDTA) test tubes for immediate analysis of haematological parameters and 2mls was put in plain containers for analysis of biochemical parameters .[12]

The animals were euthanized using sodium pentobarbital at a dosage of 800mg/kg administered intraperitoneally. Autopsy examination of all

animals was carried out and the major organs like liver, heart and kidney were surgically taken out and were fixed in 10% neutral buffered formalin.

The organ-to-body weight ratios was determined according to the expression described by Yakubu *et al* .[13]

$$\text{Relative organ weight (\%)} = \frac{\text{organ weight}}{\text{body weight}} \times 100$$

**Evaluation of haematological parameters**

Haematological parameters were measured using Sysmex XT 2000i haematology auto analyser. Validated animal specific reagents were used as guided by the veterinary laboratory at The University of Zambia.

**Evaluation of biochemical parameters**

Biochemical parameters were measured using Beckman Coulter au-480 automated chemistry analyser. Validated animal specific reagents were used as guided by the veterinary laboratory at The University of Zambia.

**Evaluation of histological parameters**

The heart, liver and kidney samples were examined for histopathological changes using a light microscope. During histological examination blinding was employed were only the researcher knew the treatment groups and dosage while the laboratory staff did not know.

**Data Analysis**

The quantitative data that was collected with regards to acute clinical toxicity signs, haematological effects, and biochemical effects was expressed as mean value and standard deviations (mean ± SD). Statistical analyses containing body weight, organ weight, haematology and biochemistry data were performed by one-way analysis of variance (ANOVA) and Levene's test for homogeneity of variance with SPSS Statistics 26.0 (IBM, Chicago, USA). A $p < 0.05$ was considered statistically significant.

**Ethical Considerations**

Ethical clearance for the study was obtained from the University of Zambia Biomedical Research Ethics Committee (REF. No. 1840-2021) and authority to conduct the research was granted by the Zambia National Health Research Authority (Ref.No. NHREB00007/30/09/2021). All laboratory work was done according to the Guidelines for Care and Use of Laboratory animals in Biomedical Research (National Research Council (US) Committee, 2011), additionally each animal was used only once, and all surviving rats were euthanized using Urethane 1000-1500mg/kg intra-peritoneal according to the American Veterinary Medicine Association guidelines [14].

**RESULTS**

**Acute Toxicity**

General clinical observations indicated no apparent signs of acute toxicity in the treatment groups when compared with the control group. Specifically, there were no cases of tremors, sedation, salivation, diarrhoea, lethargy, mydriasis, miosis, or convulsions. Additionally, no mortality was recorded during the acute phase of the study, suggesting that the extract did not induce observable acute toxic effects at the administered doses.

**Haematological Changes**

A significant increase in the white blood cell (WBC) count was observed in the treatment groups compared to the control group ($P < 0.001$, 95% CI: [21.39, 23.94]), indicating a potential immunomodulatory effect of the extract. Notably, this increase appeared to occur in a non-dose-dependent manner. Other haematological parameters, including neutrophils, lymphocytes, monocytes, eosinophils, basophils, reticulocytes, red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration

(MCHC), and platelet count (PLT), did not differ significantly between the treatment and control groups (Table 2).

significant increase in sodium (P < 0.012, 95% CI: [**134.25, 138.80**] ) and Glucose (P < 0.009, 95% CI: [**5.96, 9.47**] ) electrolytes, suggesting renal alteration in renal function.

**Table 2. Multivariate Analysis of Variance for Haematological Parameters**

| Dependent Variable | Type III SS | df | Mean Square | F | Sig. (P-value) |
|---|---|---|---|---|---|
| WBC [×10³/µl] | 67.993 | 3 | 22.664 | 10.044 | **0.001** |
| NEUT [%] | 172.158 | 3 | 57.386 | 0.953 | 0.436 |
| LYMPH [%] | 407.967 | 3 | 135.989 | 1.974 | 0.154 |
| MONO [%] | 9.373 | 3 | 3.124 | 0.782 | 0.519 |
| EO [%] | 19.098 | 3 | 6.366 | 1.252 | 0.320 |
| BASO [%] | 0.155 | 3 | 0.052 | 1.159 | 0.353 |
| RET [%] | 0.920 | 3 | 0.307 | 0.606 | 0.619 |
| RBC [×106/µl] | 2.466 | 3 | 0.822 | 1.903 | 0.165 |
| HGB [g/dL] | 7.604 | 3 | 2.535 | 1.904 | 0.165 |
| HCT [%] | 80.454 | 3 | 26.818 | 2.320 | 0.110 |
| MCV [fL] | 6.599 | 3 | 2.200 | 0.160 | 0.922 |
| MCH [pg] | 1.645 | 3 | 0.548 | 0.443 | 0.725 |
| MCHC [g/dL] | 2.005 | 3 | 0.668 | 0.683 | 0.574 |
| PLT [×10³/µl] | 338455.188 | 3 | 112818.396 | 1.300 | 0.305 |

**Table 2:** Multivariate analysis of variance (MANOVA) evaluating changes in haematological parameters across different groups. For each parameter, the Type III Sum of Squares, degrees of freedom (df), Mean Square, F-statistic (F), and significance level (Sig.) are reported. The Type III Sum of Squares measures the variance attributable to each factor after adjusting for other variables. The F-statistic tests the hypothesis of equal means across groups, and the p-value (Sig.) indicates statistical significance, with $p < 0.05$ considered significant.

**Biochemical Changes**

The extract caused a significant increase in the levels of liver enzymes aspartate aminotransferase (AST) (P < 0.005, 95% CI: [365.77, 509.23] ) and alanine aminotransferase (ALT) (P < 0.003, 95% CI: [191.22, 258.79] ), particularly in the 150 mg/kg and 600 mg/kg treatment groups. However, the increase was observed in a **non-dose-dependent** manner, suggesting a possible threshold effect or idiosyncratic response. The extract also caused

Other biochemical parameters, including total protein, albumin, electrolytes (K? , Cl? ), urea, and creatinine did not show statistically significant differences between treatment and control groups, indicating no overt hepatotoxicity or nephrotoxicity at the tested doses. (Table 3)

**Table3: Descriptive Statistics of biochemical changes across animal groups**

| Variable | Group | Mean | Std. Deviation | N | Sig(P-value) |
|---|---|---|---|---|---|
| Sodium[mmol/l] | Group A | 135.00 | 2.449 | 5 | |
| | Group B | 137.60 | 2.793 | 5 | |
| | Group C | 137.00 | .707 | 5 | |
| | Group D | 136.50 | 1.643 | 6 | |
| | Total | 136.52 | 2.112 | 21 | **0.012** |
| Chloride[mmol/l] | Group A | 101.320 | 3.4303 | 5 | |
| | Group B | 103.620 | 1.5385 | 5 | |
| | Group C | 104.340 | 1.0139 | 5 | |
| | Group D | 103.950 | 2.2224 | 6 | |
| | Total | 103.338 | 2.3809 | 21 | 0.965 |
| Urea [mmol/l] | Group A | 9.9540 | 2.23885 | 5 | |
| | Group B | 9.7840 | 1.00662 | 5 | |
| | Group C | 10.0860 | 1.62817 | 5 | |
| | Group D | 10.6333 | 1.57186 | 6 | |
| | Total | 10.1390 | 1.57070 | 21 | 0.251 |
| Creatinine[mmol/l] | Group A | 26.700 | 6.1522 | 5 | |
| | Group B | 20.800 | 5.1220 | 5 | |
| | Group C | 23.820 | 3.0663 | 5 | |
| | Group D | 23.300 | 6.1260 | 6 | |
| | Total | 23.638 | 5.3366 | 21 | 0.100 |
| Alanine Transaminase(ALT)[ IU/l] | Group A | 300 | 72.41 | 5 | |
| | Group B | 150 | 31.19 | 5 | |
| | Group C | 200 | 46.41 | 5 | |
| | Group D | 250 | 89.71 | 6 | |
| | Total | 225 | 59.93 | 21 | **0.003** |

| | | | | | |
|---|---|---|---|---|---|
| Aspartate Transaminase(AST)[IU/I] | Group A | 600 | 298.54 | 5 | |
| | Group B | 300 | 85.47 | 5 | |
| | Group C | 450 | 100.20 | 5 | |
| | Group D | 400 | 178.55 | 6 | |
| | Total | 437.5 | 165.69 | 21 | **0.005** |
| Total Protein [g/l] | Group A | 77.000 | 8.9652 | 5 | |
| | Group B | 74.240 | 5.2572 | 5 | |
| | Group C | 76.340 | 3.6143 | 5 | |
| | Group D | 67.633 | 8.7184 | 6 | |
| | Total | 73.510 | 7.6654 | 21 | 0.042 |
| Albumin[g/l] | Group A | 35.0400 | 4.94651 | 5 | |
| | Group B | 34.9600 | 2.73276 | 5 | |
| | Group C | 36.7800 | 1.94859 | 5 | |
| | Group D | 35.0533 | 4.65234 | 6 | |
| | Total | 35.4390 | 3.62616 | 21 | 0.542 |
| P-Glucose [mmol/l] | Group A | 7.8840 | 1.15099 | 5 | |
| | Group B | 6.8960 | .24946 | 5 | |
| | Group C | 7.1080 | .75291 | 5 | |
| | Group D | 8.9617 | 4.13283 | 6 | |
| | Total | 7.7719 | 2.32178 | 21 | **0.009** |

**Table 3:** Values are presented as mean ± standard deviation (SD). Group A–D represent different treatment groups. *N* denotes the number of animals per group. The *P*-value indicates the significance level from one-way ANOVA comparing means across groups. A *P*-value < 0.05 was considered statistically significant.

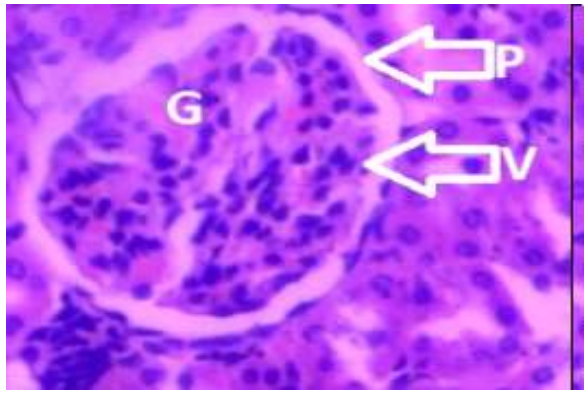
## Histopathological changes

### Effect of repeated administration of methanol root extract of *A. garckeana* on the Rat Kidney

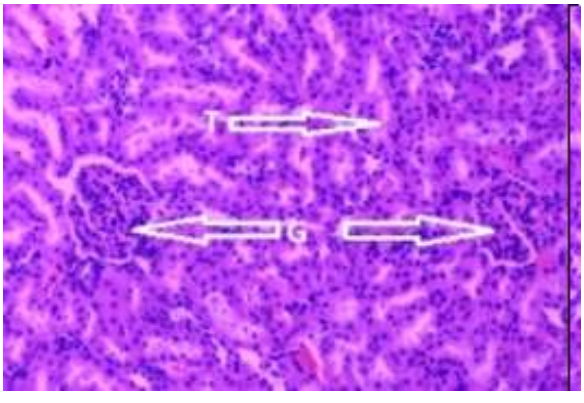
Sections of rat kidney tissue from the control group (A) exhibited normal glomeruli with tuft-like vascular structures organized into lobules of capillaries, surrounded by double-walled Bowman's capsules. These capsules featured an inner visceral layer of stellate epithelial podocytes, and an outer parietal layer lined with simple squamous epithelium, consistent with normal kidney morphology. Similarly, tissue sections from the 150 mg/kg (B) and 300 mg/kg (C) extract-treated groups displayed normal kidney architecture. The glomeruli in these groups also showed tuft-like vascular formations arranged in lobules of capillaries, enclosed by Bowman's capsules with the characteristic inner visceral layer of podocytes and an outer parietal layer of simple squamous

epithelium. The surrounding tubules, lined with cuboidal epithelial cells with eosinophilic cytoplasm and centrally placed nuclei, appeared unremarkable.

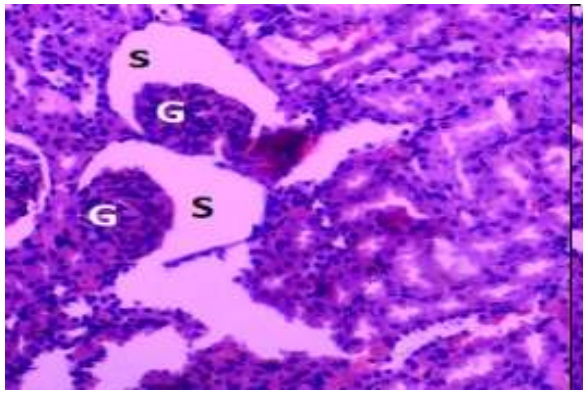
In contrast, the 600 mg/kg (D) extract-treated group revealed significant pathological changes. These samples exhibited focal segmental glomerulosclerosis (FSGS), characterized by shrunken glomeruli and widened capsular spaces. Additionally, there was evidence of focal tubular necrosis, marked by the extrusion of cytoplasm and nuclei into the lumen (Figure 1).




C




A

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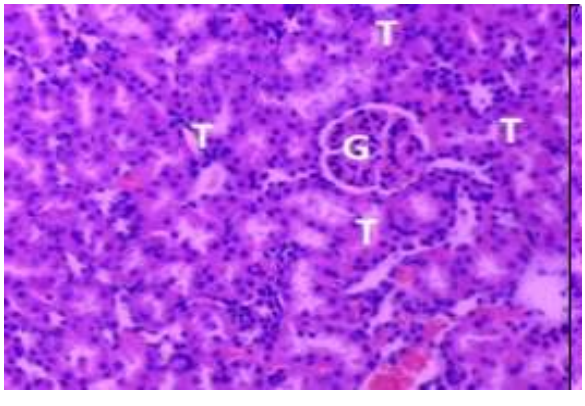



B

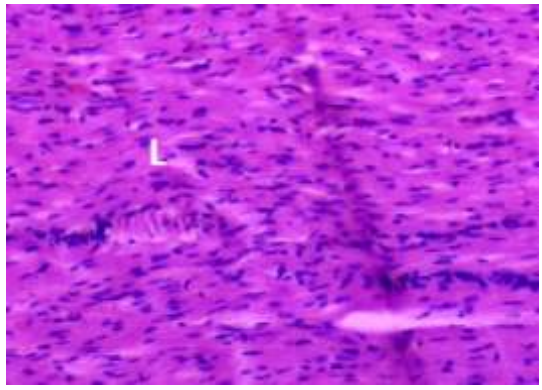
**Figure 1:** Histopathological examination of rat kidney tissues in control and extract-treated groups. **A.** (x4 Mag, H&E) Kidney tissue of control group showing normal histology with unremarkable renal tubules (T) and glomeruli (G). **B.** (x4 Mag, H&E) Kidney tissue of 150 mg/kg extract-treated rats showing unremarkable renal tubules (T) and unremarkable Glomeruli (G). **C.** (x10 Mag H&E,) Kidney tissue of 300 mg/kg extract-treated rats showing unremarkable Glomeruli (G). **D.** (x4 Mag, H&E) Kidney tissue of 600 mg/kg extract-treated rats showing ***shrunken Glomeruli (G) with widening of the capsular space (S).***

**Scale bars:** 200 μm (×4); 100 μm (×10).

**Effect of repeated administration of methanol root extract of *A. garckeana* on the Rat Heart**

Sections of the control group (A) cardiac tissues displayed normal histological characteristics. The longitudinally arranged cardiac muscle fibres were elongated, with eosinophilic cytoplasm and central, vesicular, spindle to oval nuclei. In transverse sections, these muscle fibres appeared oval, also exhibiting eosinophilic cytoplasm and central, vesicular, oval nuclei. Connective tissue fibres and their nuclei were interspersed among the muscle fibres, further confirming the normalcy of the cardiac tissue.

Similarly, sections from the cardiac tissues treated with extracts at doses of 150 mg/kg (B), 300 mg/kg (C), and 600 mg/kg (D) revealed normal longitudinally arranged cardiac muscle fibres. These fibres were elongated, with eosinophilic cytoplasm and central, vesicular, spindle to oval nuclei. The transverse sections also showed oval fibres with eosinophilic cytoplasm and central, vesicular, oval nuclei. These observations consistently confirmed that the cardiac tissues remained normal across all treated groups (Figure 2).

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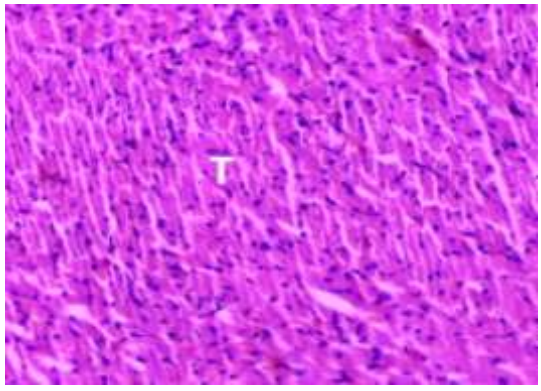


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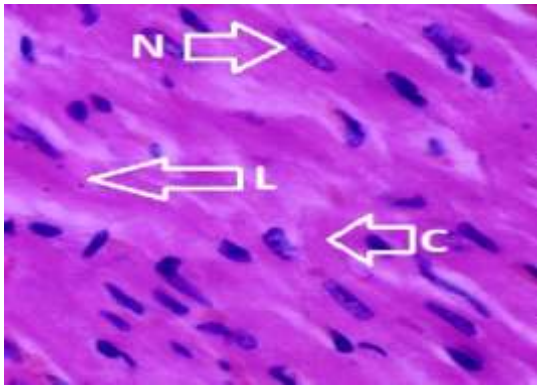
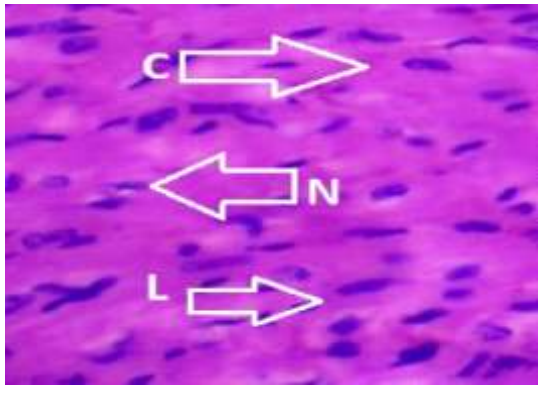



A




D

**Figure 2:** Histopathological examination of rat cardiac tissues in control and extract-treated groups. **A.** (x10 Mag, H&E) Cardiac tissue of control group showing ***unremarkable longitudinal cardiac muscle fibres(L) which are elongated and have eosinophilic cytoplasm (C) and spindle to oval nuclei (N).*** **B.** (x4 Mag, H&E) Cardiac tissue of 150 mg/kg extract-treated rats showing ***unremarkable longitudinal cardiac muscle fibres(L) which are elongated have eosinophilic cytoplasm and spindle to oval nuclei***. **C.** (x4 Mag, H&E) Cardiac tissue of 300 mg/kg extract-treated rats s***howing unremarkable transverse cardiac muscle fibres(T) which are oval have eosinophilic cytoplasm and oval nuclei. D.*** (x10 Mag, H&E) Cardiac tissue of 600 mg/kg extract-treated rats showing ***unremarkable transverse cardiac muscle fibres(T) which are oval and have eosinophilic cytoplasm (C) and oval nuclei (N).***

**Scale bars:** 200 µm (×4); 100 µm (×10).

**Effect of repeated administration of methanol root extract of *A. garckeana* on the Rat Liver**

Sections of the control group (A) liver tissues examined displayed normal liver parenchyma, characterized by sheets of polygonal hepatocytes with round, centrally located nuclei and abundant eosinophilic cytoplasm containing fine basophilic granules. These hepatocytes were organized into trabecular plates, one to two cells thick, interspersed with sinusoids lined by endothelial cells with indistinct cytoplasm and elongated nuclei. Kupffer cells were present within the sinusoids, exhibiting bean-shaped nuclei and star-shaped extensions, indicative of a healthy liver environment.

Tissue sections from the 150 mg/kg extract-treated group (B) also exhibited normal liver architecture, with hepatocytes interspersed with sinusoids that open into central veins. In contrast, sections from the 300 mg/kg extract-treated rat tissues (C) revealed signs of mild acute and subacute injury, evidenced by swelling and non-lipid cytoplasmic vacuolation of hepatocytes. Affected hepatocytes appeared distended, with centrally located nuclei displaced by clear vacuoles. Additionally, congestion of sinusoids and central veins was observed, although no signs of chronic injury or fibrosis were noted.

Chronic liver injury was identified in specimens treated with 600 mg/kg extract (D), characterized by significant lymphocytic infiltrate, perivenule lymphocyte proliferation, and fibrosis. Areas of parenchymal necrosis were present, marked by ghost hepatocytes with indistinct borders and inconspicuous nuclei (Figure 3).

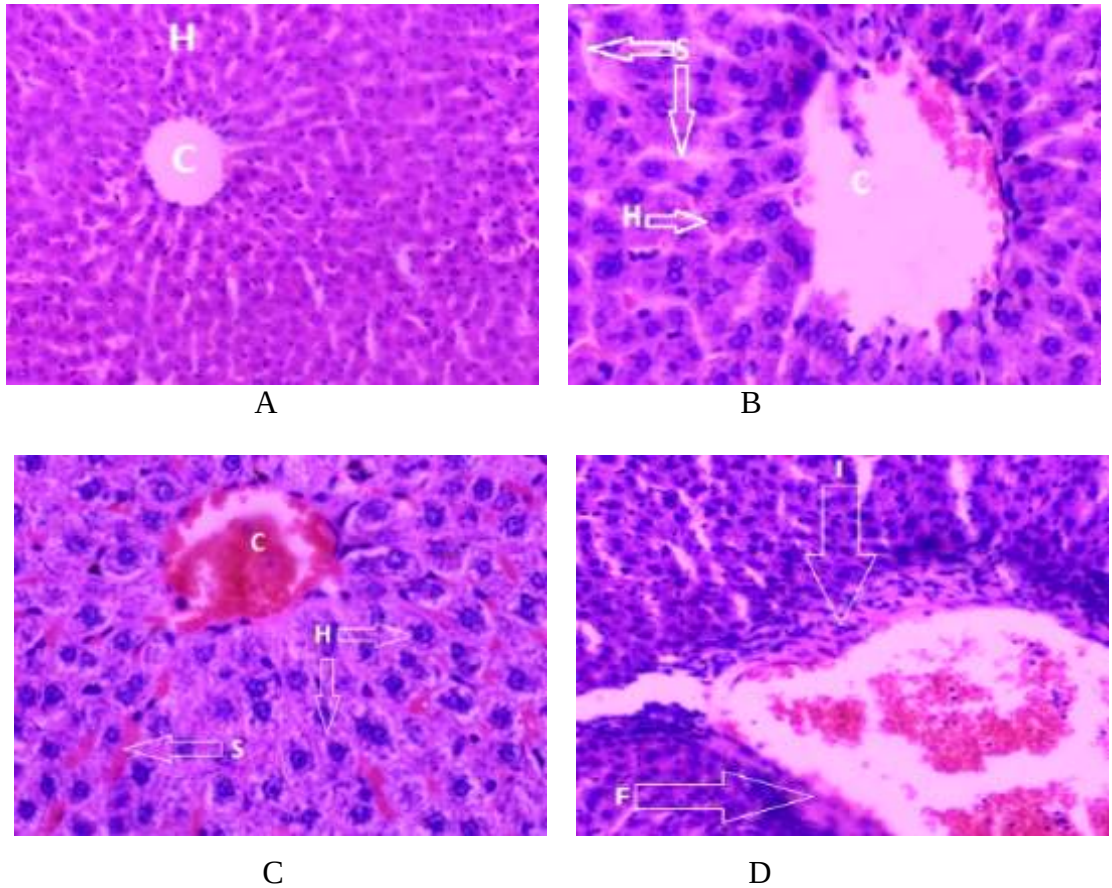



**Figure 3:** Histopathological examination of liver tissues in control and extract-treated groups. **A. *(x4 Mag,*** H&E***)*** Liver tissue of the control group ***showing normal hepatocytes(H) that is polygonal in shape with round, centrally located nuclei and abundant eosinophilic cytoplasm with sinusoids which open into central veins (C)in a control group specimen. B. (x10 Mag,*** H&E***) Liver tissue of*** 150 mg/kg

extract-treated rats showing ***normal hepatocytes(H) that is polygonal in shape with round, centrally located nuclei and abundant eosinophilic cytoplasm with sinusoids (S) which open into central veins (C). C. (x10 Mag,*** H&E***) Liver tissue of*** 300 mg/kg extract-treated rats showing ***swollen hepatocytes (H) that are round in shape with a stellate, centrally located nuclei and vacuolated cytoplasm. Central vein(C) and Sinusoids (S) show marked RBC congestion. D . (x10 Mag,*** H&E***) Liver tissue of*** 600 mg/kg extract-treated rats showing ***Periportal inflammation (I) and Periportal fibrosis (F).***

**Scale bars:** 200 μm (×4); 100 μm (×10).

## DISCUSSION

In determining the acute clinical toxicity, our study showed that there were no apparent signs of clinical toxicity like tremors, sedation, salivation, diarrhoea, lethargy, mydriasis, miosis, or convulsions in any of the treatment groups (150 mg/kg, 300 mg/kg, and 600 mg/kg) or the control group. This could be attributed to the fact that the medium lethal dose for *A. garckeana* was >5000 mg/kg, as evidenced by oral administration of *A. garckeana* extract to rabbits in a study by Itodo *et al*., which indicated no toxic signs or mortality at a dose of 5000 mg/kg.[15] When compared to other plants in the family (*Malvaceae*), these results were consistent with findings from Pongri *et al.*, which indicated that administration of aqueous extract of *Grewia mollis* stem bark at a dose of 9600 mg/kg produced no adverse effects or signs of toxicity such as changes in the skin, fur, eyes, mucous membranes, tremors, salivation, or diarrhoea up to 14 days of observation.[16] These findings were also corroborated by other studies on Malvaceae species.[17,18]

For haematological parameters, our study showed a significant increase ($P<0.001$) in white blood cell (WBC) count in the treatment groups compared to the control group. Interestingly, the WBC elevation occurred in a non-dose-dependent manner. The effect may be attributed to glycosides present in the *A. garckeana* extract,[11] compounds known for their anti-inflammatory properties and modulation of immune responses in pathological conditions such as sexually transmitted infections and malaria. These results are consistent with the study by Pongri *et al*., where administration of *Grewia mollis* extract elevated granulocytes and monocytes non-dose-dependently,[16] as well as findings by Sumanta et al. following administration of *Hibiscus rosa-sinensis* extracts.[17]

For biochemical parameters, our study found a non-significant fluctuation in creatinine levels between treated and control groups, suggesting no impairment of renal function. These results contrast with those reported by Yusuf *et al.*, where a significant and dose-dependent decrease in serum creatinine suggested renal dysfunction.[11] The difference may arise from variations in dosing frequency, as our study employed daily dosing (OECD 407 protocol) while Yusuf *et al*. used twice-daily dosing, likely increasing cumulative nephrotoxic effects. Regarding liver function, our study demonstrated significant ($P<0.01$) elevations in liver enzymes (ALT and AST) at both 150 mg/kg and 600 mg/kg doses, though without a clear dose-dependent trend. This **non-dose-dependent response** suggests that hepatocellular stress may occur even at lower doses, possibly due to early mitochondrial injury, generation of reactive oxygen species (ROS), or saturation of hepatic detoxification pathways. It may also reflect interindividual variability among test animals or threshold effects where certain doses initiate hepatocellular injury, but escalation beyond a point does not linearly increase enzyme levels.

**Mechanistically**, the hepatotoxicity observed could involve oxidative stress from phenolic compounds and flavonoids present in the extract, leading to lipid peroxidation, mitochondrial dysfunction, and direct hepatocyte injury. Reactive metabolites generated during phytochemical metabolism may covalently bind to cellular proteins, triggering inflammatory cascades (e.g., TNF-α, IL-6 upregulation) and

apoptosis. Similarly, nephrotoxic effects, particularly glomerular shrinkage and tubular necrosis, could result from endothelial injury, oxidative damage, and ischemic effects secondary to vascular congestion.

Histopathological analysis of the kidneys revealed no abnormalities at 150 mg/kg and 300 mg/kg; however, the 600 mg/kg group exhibited focal segmental glomerulosclerosis (FSGS) and focal tubular necrosis, features consistent with toxic nephropathy. These findings indicate a **dose-dependent nephrotoxic effect** following prolonged administration of the extract, corroborating results from Amgad *et al*. and Yemele *et al.* on related *Malvaceae* plants.[19,24] In contrast, histopathological examination of cardiac tissue across all groups revealed no abnormalities. This finding suggests minimal cardiotoxicity, possibly because cardiac tissues are less involved in the metabolism and clearance of phytochemicals compared to the liver and kidneys.

Liver histopathology varied with dose, In the 150 mg/kg group, hepatocytes appeared normal, indicating **reversible or minimal** injury. In the 300 mg/kg group, hepatocytes showed cytoplasmic vacuolation, sinusoidal congestion, and swollen cells with stellate nuclei changes that are typically **reversible if exposure ceases early**. At 600 mg/kg, features included periportal inflammation, fibrosis, and periportal chronic inflammation, indicating **irreversible hepatic injury**, consistent with chronic hepatitis and hepatic fibrosis. Thus, **reversible toxicity features** (cytoplasmic vacuolation, mild congestion) were noted at moderate doses, while **irreversible damage** (fibrosis, chronic inflammation) dominated at high doses.

These findings have important implications, **For traditional medicine**, although *A. garckeana* appears safe at lower doses, prolonged use or high-dose administration carries significant hepatotoxic and nephrotoxic risks. Traditional practitioners should be advised to **limit dosage and treatment duration** to avoid latent toxicity. **In regulatory toxicology**, our results highlight the necessity of **chronic toxicity evaluations** for herbal extracts, beyond acute toxicity studies. Standardization of active phytochemicals, phytometabolite profiling, and labelling of potential adverse effects should be mandatory for herbal formulations derived from *A. garckeana*. Overall, while *A. garckeana* demonstrates therapeutic potential, cautious use and strict monitoring are essential to prevent long-term organ damage.

**Limitations**

The study had limitations, including the lack of baseline haematological and biochemical data due to small animal size, use of a single species without sex distribution analysis, and absence of longer-term toxicity or recovery-phase assessments, which may restrict the generalizability and completeness of the toxicity profile. Future studies addressing these gaps will be critical for better defining the safety margins of *A. garckeana* use.

## CONCLUSION

The primary objective of this study was to evaluate the acute and subacute toxicity of the methanol root extract of *A. garckeana* in albino rats. Based on the findings, it can be concluded that repeated administration of the extract induced subacute toxicity in the liver and kidneys, as evidenced by histopathological changes. The observed toxicity was dose dependent. However, during the acute phase (14 days) of administration, no clinical signs of acute toxicity were observed, suggesting that the extract may be safe for short-term use. These findings suggest that **prolonged use may pose risks** to renal and hepatic function

- **What is already known on this topic:**

  *A. garckeana* causes alterations in serum creatinine signifying renal toxicity in Wistar rats.

- **What this study adds:**

  The current study showed the acute and subacute toxicity profile for *A.garckeana* methanol root extract. Investigation of the

clinical toxicity signs, biochemical, haematological and histopathological alterations caused by *A. garckeana* .

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### Competing Interests

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

### Authors' Contributions

All the listed authors have made a significant scientific contribution to the research in the manuscript. **Eugene Kanta:** Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original draft. **Semenova Musalwa-Muyangwa:** Validation, Supervision, writing – reviewing and editing. **Rehana Omar:** Validation, writing – reviewing and editing **Lavina Prashar:** Validation, Supervision, writing – reviewing and editing.

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