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Research Article

Toxicological Assessment of Aqueous Leaf Extract of *Anisopus mannii*: Acute and Sub-Acute Studies in Albino Rats

Hauwa M. S. Haruna[1], Hauwa Umar[1], Amina Zakari[1] and *Gabi Baba[1,2]

[1]Department of Applied Chemistry, Kaduna Polytechnic, Kaduna, Nigeria
[2]Department of Biochemistry, Kaduna State University (KASU), Kaduna, Nigeria
***Corresponding Author's email*: babagabi1969@gmail.com; Phone:** +2348025255143

ABSTRACT

The study investigated the acute and sub-acute toxicity of aqueous leaf extract of *Anisopus mannii* in albino rats to assess its safety profile and potential organ-specific effects. Acute toxicity was evaluated using Organization for Economic Cooperation and Development (OECD) guideline 401, with doses ranging from 1000 to 5000 mg/kg administered orally. The rats were observed for behavioral changes, toxicity symptoms, and mortality over 14 days. For the sub-acute study, 20 male rats were divided into four groups and administered 200, 400, 600 mg/kg of the extract, or distilled water (control) daily for 28 days. Biochemical markers of liver and kidney function were analyzed, and histopathological examinations of liver and kidney tissues were performed. The acute study revealed no mortality nor significant behavioral changes ($p \geq 0.05$), with an LD50 value greater than 5000 mg/kg, indicating low toxicity. Sub-acute analysis showed a dose-dependent increase in urea and creatinine levels, suggesting potential renal stress at higher doses. However, liver function markers, including total bilirubin, conjugated bilirubin, ALT, and AST, were significantly reduced ($p \leq 0.05$), indicating hepato-protective effects. Histopathological examination of the liver showed intact hepatic architecture with no necrosis or inflammation, while the kidneys exhibited mild alterations. These findings suggest that *Anisopus mannii* extract is relatively safe at therapeutic doses but may pose renal risks at higher concentrations. Further studies are recommended to elucidate the mechanisms of action and establish long-term safety profiles.

Keywords: *Anisopus mannii*; Toxicity; Acute, Sub-acute; Biochemical markers; Histology; Albino rats

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INTRODUCTION

Medicinal plants have long been recognized for their therapeutic potential and are widely used in traditional folkloric medicine across the world. However, with the recent growing global interest in herbal medicine, there is an increasing demand for scientific validation of the efficacy and safety of these herbs (Omonkhelin *et al*, 2007; Venkatalakshion, 2012). Toxicological assessment is a crucial step in ensuring that plant-based treatments are safe for human consumption. Among the numerous medicinal plants, *Anisopus mannii* (family *Apocynaceae*) has garnered attention for its reported medicinal properties. Traditionally, this plant is used in West Africa for its claimed anti-diabetic, anti-inflammatory, and analgesic effects. However, despite its ethno-botanical significance, scientific studies exploring the safety profile of *A. mannii* are limited. Hence, evaluating the acute and sub-acute toxicity of its extract is essential for validating its therapeutic potential and safety.

Anisopus mannii is used in traditional medicine for a variety of conditions, including diabetes and pain management (Etuk *et al*., 2009). Phytochemical analyses reveal that *A. mannii* leaves contain bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins, which are associated with its pharmacological effects (Nwafor *et al*., 2013). However, these bio-active compounds may also carry risks, as they can cause organ toxicity and adverse

biological responses. Flavonoids, in some instnaces, are known for their antioxidant properties, but at high doses, they may cause oxidative stress, particularly in individuals with compromised health (Zhou *et al*., 2019). Additionally, alkaloids have been reported to produce both beneficial and toxic effects, depending on their concentration and the duration of exposure (Saidu *et al*., 2017).

Toxicity testing in rats allows for the observation of clinical signs, blood chemistry changes, and histopathological effects of the plant extract on vital organs, including the liver, kidneys, and heart. Acute and sub-acute toxicity assessments in albino rats also help in identifying safe dosages and understanding the mechanisms of toxicity in vivo (Santos *et al*., 2016).

This study is therefore designed to evaluate the acute and sub-acute toxicological effects of *A. mannii* leaf extract in albino rats, using the lethal dose (LD_{50}) as the index and assessing the physiological and histo-pathological responses of rats following oral administration of the extract.

MATERIALS AND METHODS

Sample Collection and Identification

Fresh leaves of *A. mannii* were collected in December 2022 from Unguwan Rimi farm, Kaduna, Nigeria. The plant material was identified and authenticated by a botanist in the Herbarium Unit, Department of Botany, Kaduna State University (KASU), Kaduna, Nigeria. A voucher specimen with the number 217 was deposited for future reference.

Animals Used in the Study

Male Wistar rats, weighing between 150 and 200 g, were used for this study. The animals were obtained from Biological garden, Animal House Unit, of Kaduna Polytechnic, Tudun wada, in Kaduna, Nigeria, and acclimatized to laboratory conditions for 14 days prior to the commencement of the experiments. Six rats were housed per cage under well-ventilated conditions, maintained at a temperature of 25–27°C with a 12-hour light/dark cycle. The animals were provided with unrestricted access to rodent pellets and potable water *ad libitum*. Cage bedding and water bottles were cleaned daily. All animal handling and experimental procedures were conducted in compliance with the International guidelines for the care and use of experimental animals.

Acute Toxicity Study

The oral acute toxicity test was conducted following the Organization for Economic Cooperation and Development (OECD) Guideline 401 for the testing of chemicals. Male Wistar rats (150–200 g) were used, and the study was carried out in two phases. In the first phase, three groups of rats (three rats per group) were administered oral doses of 1000, 1500, and 2000 mg/kg of *A. mannii* leaf extract. The animals were observed for signs of toxicity and mortality over a 24-hour period, with particular attention during the first four hours.

In the second phase, three additional groups of rats were orally administered higher doses of 3000, 4000, and 5000 mg/kg of the extract. These rats were monitored for signs of toxicity using the same observation protocol and were further evaluated daily for seven days. Observations focused on clinical signs such as salivation, paw-licking, writhing, changes in body weight, and other indicators of toxicity.

Sub-Acute Toxicity Study

For the sub-acute toxicity assessment, twenty rats were weighed and randomly assigned to four groups, with five rats per group. The groups were treated with *A. mannii* leaf extract at daily oral doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg, while the control group received distilled water. Observations for toxicity signs and changes in body weight were recorded throughout the study period. The animals were treated for 28 days, starved over night and were euthanize on the 29th day to collect blood samples via cardiac puncture under mild anesthesia, for biochemical analysis and organs collection for histopathology.

Evaluation of liver and kidney function indices

The blood was collected in a plain sample bottle as described earlier, allowed to coagulate, then centrifuge at 3000 rpm for 10 minutes to separate the serum. The serum obtained were used to evaluate the levels of alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), and conjugated bilirubin (CBIL). The Kidney function indices were evaluated by measuring the serum levels of electrolytes, urea, and creatinine,

Histopathological examination of organs

Organ collection and fixation:

After euthanizing the animals on day 29 of treatment, the liver and kidneys were carefully dissected and collected. The organs were fixed in 10% neutral buffered formalin for a minimum of 24 hours to preserve tissue integrity. For tissue processing and staining, the fixed tissues were dehydrated through a graded series of alcohol, cleared with xylene, and embedded in paraffin wax. Thin tissue sections (4–5 µm) were cut using a microtome and stained with hematoxylin and eosin (H&E) to enhance microscopic visualization. The stained sections are examined under a light microscope to identify any structural abnormalities or signs of damage, such as necrosis, inflammation, or cellular degeneration.

Data Analysis

Data were analyzed using SPSS software version 21. Results were expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple comparison test to determine significant differences between groups. Statistical significance was considered at a $P < 0.05$. Group results were presented as mean ± SD for sample sizes of $n = 5$ and $n = 3$, as appropriate.

RESULTS

The percentage yield is presented in Table 1, a yield of 19.5% moderately high which reflects an efficient extraction process, suggesting the suitability of water and suggests that *A. mannii* contains a considerable amount of water-soluble compounds.

Acute Oral Toxicity Study

The results of the general behavioural changes, symptoms of toxicity, and mortality observed in animals treated with *Anisopus mannii* leaf extract is presented in Table 2. The Behavioural Parameters such as Locomotion, Reaction to noise, touch response, skin fur, and feces were reported as "normal" (N) across all animal groups, indicating no adverse effects or abnormal behaviours were observed in these aspects. Sleep, convulsion, and tremor parameters were noted as "absent" (A) for all animals, suggesting that the extract did not induce sedation, seizures, or involuntary muscle movements, while no mortality was recorded in any of the animals after 48 hours or 14 days post-treatment, indicating the absence of lethal effects at the tested doses. The LD_{50} value was 0 mg/kg, which signifies that no lethal dose was determined within the tested dosage range, as no mortality occurred.

Effect of *Anisopus Mannii* on Urea, Creatinine and Electrolytes Level of the Treated Rats and Control.

The effect of *A. Mannii* on electrolytes level is presented in Table 3 with the levels of Sodium (130–150 mmol/L) Potassium (2.4–6.3 mmol/L) across all groups (low, medium, high doses) remained within the normal range , indicating that *A. mannii* extract had no significant effect ($p \geq 0.05$) on both sodium and potassium homeostasis. Chloride (95–110 mmol/L) and Bicarbonate (24–32 mmol/L) levels were stable and within the normal range across all treatment groups, showing no disturbances in acid-base balance or chloride and bicarbonate regulation. Urea levels increased with higher doses of *A. mannii* extract, with the high-dose group showing the most significant elevation (4.70 ± 1.23 mmol/L) ($p \leq 0.05$). Although still within the normal range (2.4–6.3 mmol/L), this rise suggests that high doses may impose mild renal stress, possibly affecting glomerular filtration. Creatinine on the other hand showed slight fluctuations across the treatment groups but remained within the normal range (9–126 µmol/L). The low and high-dose groups exhibited mild increases compared to the control, which might suggest mild renal compromise at these doses.

The Table 4 summarizes the effect of *A. mannii* leaf extract on liver function parameters in albino rats treated with low, medium, and high doses compared to the normal control group. The total protein levels increased progressively across the treatment groups, with the high-dose group nearing the upper normal range (79.00 ± 3.74 g/L).Albumin levels were relatively stable and within the normal range (35–50 g/L) across all groups. ALT and AST levels remained within the normal range (0–12 m/L) in all groups, with only minor variations. ALP levels, however, showed a slight dose-dependent increase across the treated groups but remained within the normal range. Both TBIL and CBIL levels significantly decreased ($p \leq 0.05$) in treated groups compared to the control, with the medium and high-dose groups showing the lowest values.

Table 5 highlights the hematological effects of *A. mannii* aqueous extract on albino rats treated with low, medium, and high doses. The PCV levels range from 37.11 ± 0.08% (medium dose) to 40.31 ± 1.20% (high dose). These values are within the physiological range for rats, indicating that *A. mannii* does not induce anemia or compromise red blood cell production. WBC levels decreased slightly in the medium dose group (4.85 ± 1.21 cells/µL) compared to the low dose (6.11 ± 0.72 cells/µL) but remained relatively stable in the high dose group (5.03 ± 0.92 cells/µL). Neutrophil levels are slightly lower in the medium dose group (55.18 ± 0.33%) compared to the low and high dose groups (59.21 ± 0.69% and 60.22 ± 1.42%, respectively). Lymphocyte levels increased progressively across the doses, with the high-dose group recording the highest value (36.11 ± 0.87%).

Table 1: The percentage yield of aqueous extracts of *Guiera senegalensis*

Plant	Percentage Yield (%)
Anisopus manni	19.5%

Table 2: General behavioural changes, symptoms of toxicity and mortality after treatment with Aqueous Leaf Extracts of *Anisopus mannii*

Parameters studies	***Anisopus mannii***		
Animal number	1	2	3
Locomotion	N	N	N
Reaction to noise	N	N	N
Touch response	N	N	N
Sleep	A	A	A
Convulsion	A	A	A
Tremor	A	A	A
Skin fur	N	N	N
Feaces	N	N	N
Mortality after 48h	0	0	0
Mortality after 14days	0	0	0
LD50(mg/kg)	0	0	0

N: Normal; A: Absent; 0: No mortality

Table 3: Kidney function and electrolytes for assessment of rat treated with different doses of *Anisopus Manni* extract and the control

Parameter	**Normal Control**	**A. *manni***		
		Low dose	**Medium dose**	**High dose**
Sodium (Na^+) 130 – 150mmo/L	138.33±2.34	613.60±2.07	134.80±4.09	135.25±1.25
Potassium (K^+) 2.4 – 6.3mmol/L	4.20±0.60	3.70±0.19	3.62±0.23	3.80±0.29
Chloride (Cl^-) 95 – 110mmol/L	96.67±2.07	96.80±1.48	96.60±1.67	97.00±1.83
Bicarbonate (HCO_3) 24 – 32mmol/L	25.17±1.17	25.60±1.82	25.80±1.30	25.25±1.50
Urea 2.4 – 6.3mmol/L	2.62±0.44	2.92±0.63	2.48±1.57	4.70±1.23
Creatinine 9 – 126mmol/L	36.50±2.95	46.60±2.58	28.40±3.20	31.75±4.27

Table 4: Effect of *Anisopus mannii* on Liver function parameters of Rats treated with different doses

Parameter	**Normal Control**	***A. manni***		
		Low dose	**Medium dose**	**High dose**
Total Protein (58 – 80g/L)	63.67±5.75	70.20±1.64	76.20±4.82	79.00±3.74
Albumin (35 – 50g/L)	35.50±2.51	35.80±1.92	35.80±2.86	37.75±2.63
Alanine transaminase (0 – 12m/L)	11.33±1.21	11.40±2.97	11.60±2.07	11.75±2.22
Aspartate transaminase (0 – 12m/L)	11.67±1.03	10.60±3.85	11.00±0.67	12.00±0.82
Alkaline phosphatase	67.50±4.09	74.60±6.9	73.00±18.19	75.25±13.05
Total bilirubin (1.7 – 17mmol/L)	11.00±1.41	3.4±1.1	2.2±03	3.6±1.3
Conjugated Bilirubin (1.7 – 8.5mmol/L)	6.50±2.12	2.0±0.3	1.7±0.7	1.8±0.4

Table 5: Effect of *Anisopus mannii* aqueous extracts on hematological parameters of the treated rat at different doses over the period of the treatment

Parameter	*A. manni*		
	Low dose	Medium dose	High dose
Packed Cell Volume(PCV) %	38.11±0.01	37.11±0.08	40.31±1.20
White Blood Cell (WBC) (cells/uL)	6.11±0.72	4.85±1.21	5.03±0.92
Neutrophils (N)%	59.21±0.69	55.18±0.33	60.22±1.42
Lymphocytes(L)%	27.81±0.02	32.34±0.04	36.11±0.87

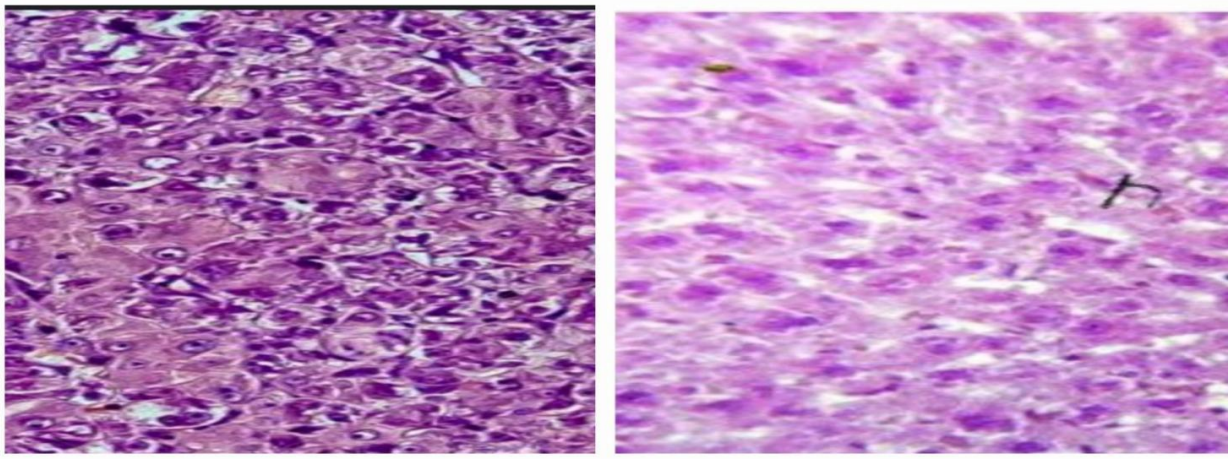

Plate 1: The micro-graph X100 of liver sections of normal group and highest doses (600mg/kg) of *A. mannii* (H&E Staining) Showing normal hepatic cells.

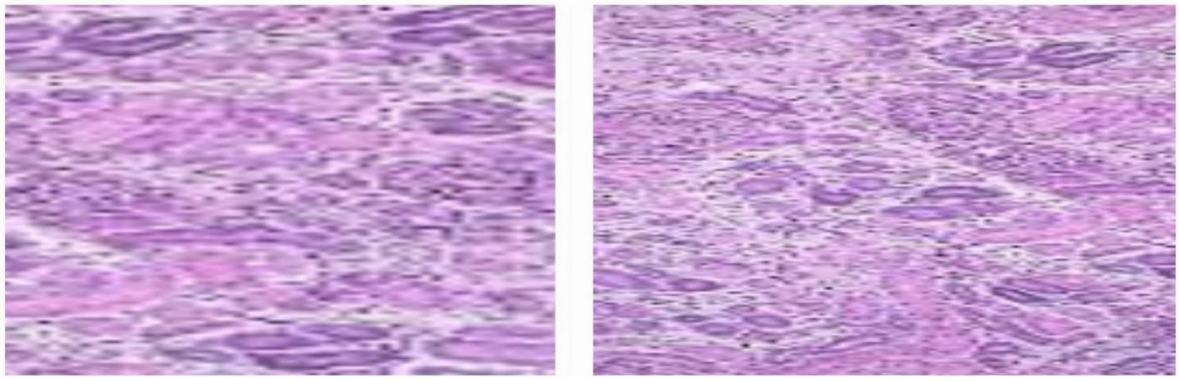

Plate 2: : The micro-graph X 100 of the Histology of kidney tissue of A. Normal group and B. albino rat group treated with 600mg/kg body weight of aqueous leaves extracts of *A. manni* after 28days. The glomerulose proximal tubule, distal tubule at intact positron A &B. Distortion and measure of fats/or crystal were assent on B

DISCUSSION

Nature has been a source of medicinal agents for thousands of years and impressive number of modern drugs have been isolated from natural sources (Venkatalakshion, 2012). This plant based traditional medicinal system continues to play essential roles in health care, with about 80% of the world` inhabitants relying mainly on traditional medicines for their primary health care especially in rural areas (Omonkhelin *et al.*, 2007). The *A. mannii* extract was prepared and stored at suitable temperatures to preserve the quality and quantity of the phyto-constituants within the plant. According to Makkar (2000) the moisture content of fresh plant material changes the chemical composition and properties of the plant over time. Grinding of the plant material into fine particles was to create a larger surface area to allow higher extraction of bio-active compounds. The high percentage yield (w/w) of 19.5% was due to high presence of more polar compounds *in A. manni.* Parekh *et al.* (2005) maintains that these observations can be explained due to the polarity of the solvent of extraction. Hence, the extract yield increased when solvents of polarity were used. The principal aim of evaluating the safety of any medicinal plant is to

identify the nature and significance of adverse effect and to establish the exposure level at which the toxicity effect or safety level is observed. The acute toxicity study indicated that the aqueous extract of *A. mannii* administered through oral route using the up and down method of acute toxicity testing did not produce any sign of toxicity, nor death in the experimental animals tested. According to OECD criteria under its Globally Harmonized Classification System (GHS) for chemical substances and mixtures, with $LD_{50} \geq 2000–5000$ mg/kg are categorized as unclassified or category 5 (Organization for Economic Development, 2001). This suggests that the oral LD_{50} of the plant being greater than 5000 mg/kg and relatively safe.

The kidney as an excretory organ is central to the normal functioning of the body. Its role in maintenance of the body homeostasis, excretion of waste products of metabolism, drugs and chemical are vital to maintenance of health (Okolie, 2011). Among the waste products of metabolism excreted by the kidney are urea and creatinine, while in the tubule's electrolytes are reabsorbed in maintenance of body's homeostasis. Creatinine and urea are non-protein nitrogenous metabolites that are cleared by the body following glomerulous filtration, thus assessment of serum urea, creatinine and electrolytes (Na^+, K^+, Cl^-, HCO_3^-) are vital and sensitive biochemical markers which are usually employed in the diagnosis of renal failure and damage (Yakubu *et al.,* 2003; Tietz, 2008; Agbasi *et al.,* 2010). The major non-protein nitrogenous catabolite of protein metabolism is urea. Creatinine is produced endogenously in the muscle by a non-enzymic action on creatine phosphate (Chatterjea and Sinde, 2012). The electrolytes, urea and creatinine are markers of kidney functions. Thus result in Table 3 indicates that *A. mannii* extract has minimal effects on electrolyte balance, maintaining normal sodium, potassium, chloride, and bicarbonate levels across all doses. However, the increase in urea levels at higher doses suggests possible renal stress or reduced excretion efficiency of the kidney. Moreover, the observed mild elevation of creatinine in some treated groups which further supports the observed possible renal stress, even though the obtained values of the tested kidney function parameters remain within the physiological range. The findings suggest that while *A. mannii* extract does not significantly ($p \geq 0.05$) disturb electrolyte homeostasis, higher doses may induce mild renal effects, particularly in urea metabolism. These observations are consistent with other studies on plant-based extracts that report dose-dependent renal effects (Olorunnisola *et al.*, 2012).

The role of liver is important for survival of animals. Their functionality can be measured by serum biochemical analysis, which are crucial in the toxicological evaluation of xenobiotics. Serum liver function tests provide information about the status of the liver. The liver enzymes (aminotransferases; ALT and AST) describe its cellular integrity, while albumin and total protein levels describe its functionality. These liver enzymes (AST and ALT) are principally produced by the liver cells and any adverse effect on the liver may lead to an increase in the serum level of these enzymes (Brautbar and Williams, 2002; Bariweni *et al*., 2018). Hence high levels of liver enzymes are signs of hepato-cellular toxicity, whereas a decrease may indicate enzyme inhibition. However, ALT is the most sensitive marker of liver damage or toxicity since AST is also found in abundance in kidneys, testes, cardiac and skeletal muscles (Friedman *et al*., 1996; Bariweni *et al*., 2018). In this study, the results demonstrated that *A. mannii* extract has a favorable effect on liver function, enhancing protein synthesis and bilirubin metabolism while not inducing liver damage as evidenced by the stability of the liver enzymes results ALT, AST, and ALP levels (Adeoye *et al*., 2016). The decrease in bilirubin levels highlights the potential of *A. mannii* to improve liver detoxification functions.

The findings suggest that *A. mannii* extract is probably hepato-protective and does not compromise liver integrity, even at high doses. These results align with previous studies on plant-based extracts demonstrating hepatoprotective effects through antioxidant or anti-inflammatory mechanisms (Olorunnisola *et al*., 2012; Adeoye et al., 2016). However, further studies are necessary to elucidate the biochemical pathways involved and the extract hepato-protective activity

Haematopoiesis is the process of blood cell formation. Changes in the haematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies. All blood cells are believed to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet) (Sulaiman *et al*., 2015). In this study, the hematological parameters in Table 5 showed that *A. mannii* extract has a stabilizing or mildly stimulatory effect on erythropoiesis and immune cell profiles without inducing cytotoxic effects. The increase in lymphocyte percentage, particularly at higher doses, suggests an immuno-modulatory potential that could enhance adaptive immunity. The findings suggest that *A. mannii* aqueous extract is haematologically safe and may exert mild immuno-stimulatory effects, especially at higher doses. These results are consistent with earlier studies on plant-based extracts exhibiting positive effects on hematological parameters (Yakubu *et al*., 2008; Sulaiman *et al*., 2015). Further research is needed

to elucidate the mechanisms of its hematological impact. Histopathological examinations of both kidney and liver sections of the normal control group and the groups treated with *A. mannii* as presented in Plate I and II showed normal hepatocytes and veins with uniformity in cells. The extract fractions presented their hepato-protective effects and preserved the normal texture of the kidney and liver cells with no major cellular alterations.

CONCLUSION

The results of this study indicate that the aqueous leaf extract of *A. mannii* is largely non-toxic, even at higher doses, as evidenced by the biochemical, hematological, and histological findings. Absence of significant adverse effects across these parameters indicated that *A. mannii* aqueous leaf extract is safe and does not cause acute or sub-acute toxicity in albino rats. However, further studies, including chronic toxicity testing and clinical trials, are recommended to confirm its long-term safety and efficacy.

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