

Acute and Sub-acute Toxicity Studies of *Senna alata* on Mice and Wistar Rats

Rasheedat T. Baba^{1*}, Rasaq A. Salaudeen¹, Maryam Aladodo², Zainab A. Dangana¹, Zainab K. Muhammad¹, Hawawu B. Sowole¹, Hussaina U. Sani¹, Firdaus Yakubu¹.

¹Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Al- Hikmah University, Ilorin, Kwara State, Nigeria.

²Department of Immunology, Faculty of Nursing and Allied Health Sciences, University of Abuja, Nigeria.

ABSTRACT

This study investigated the acute and subacute toxicological effects of aqueous and methanol leaf extracts of *Senna alata* in experimental animals. For the acute toxicity assessment, 25 mice were divided into five groups and administered oral doses of 2,000 or 3,000 mg/kg body weight (bw) of either aqueous (AES) or methanol (MES) extract. In the 28-day subacute study, 36 Wistar rats received daily oral doses of 500, 1,000, 2,000, or 3,000 mg/kg bw of AES or MES. The extraction yields were 6.2% for AES and 7.9% for MES. Biochemical and haematological indices, together with liver and kidney histopathology, were evaluated. No mortality, abnormal behaviour, or other overt signs of toxicity were observed, and the median lethal dose (LD₅₀) of both extracts exceeded 3,000 mg/kg. Body weight and relative organ weights did not differ significantly between treated and control animals. Serum alanine aminotransferase (ALT) increased from 30.3 IU/L in controls to 39.4 and 43.5 IU/L following 2,000 mg/kg AES and MES, respectively, and to 51.6 and 54.8 IU/L at 3,000 mg/kg. Aspartate aminotransferase (AST) similarly increased from 57.3 IU/L in controls to 67.1 and 68.5 IU/L at 2,000 mg/kg and 73.1 and 79.5 IU/L at 3,000 mg/kg ($p < 0.05$). Other biochemical indices remained largely unchanged. Haematological parameters were generally unaffected, although platelet counts increased significantly at higher doses ($p < 0.05$). Renal histology remained normal, whereas high-dose treatment produced mild hepatic sinusoidal dilation and portal inflammation. The extracts therefore exhibited low acute toxicity, although prolonged or high-dose exposure may induce mild hepatic stress.

Keywords: *Senna alata*, Toxicity, Aqueous, Methanol, Extract.

Received 29 July 2026

Revised 08 October 2026

Accepted 09 October 2026

Published online: 19 October 2026

Copyright: © 2026 Baba *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Medicinal plants continue to serve as an important source of therapeutic agents for the prevention and treatment of diseases worldwide.¹ In many developing countries, traditional medicine remains a major component of healthcare delivery, with herbal remedies being widely utilised because of their accessibility, affordability, and perceived effectiveness.² Despite their extensive use, the safety profiles of numerous medicinal plants have not been fully established through comprehensive toxicological investigations, making safety evaluation an essential aspect of herbal medicine research.³

Senna alata (L.) Roxb. (synonym: *Cassia alata*) is a medicinal plant belonging to the family Fabaceae and is widely distributed throughout tropical and subtropical regions of Africa, Asia, and South America.⁴ The plant has traditionally been used in the treatment of fungal infections, skin diseases, constipation, malaria, diabetes mellitus, and inflammatory conditions.⁵ Its therapeutic activities have been attributed to the presence of several bioactive phytochemicals, including anthraquinones, flavonoids, alkaloids, tannins, saponins, and phenolic compounds.⁶

Although herbal medicines are generally considered safe because of their natural origin, some phytochemical constituents may produce adverse effects when consumed in excessive quantities or over prolonged periods.⁷ Toxic substances present in medicinal plants may affect vital organs such as the liver, kidneys, and haematopoietic system, thereby posing potential health risks to users.⁸ Consequently, scientific assessment of the toxicity profile of medicinal plants is necessary to ensure their safe and effective use.

Toxicity studies are fundamental in the evaluation of medicinal plants and herbal products. Acute toxicity studies assess the harmful effects that may occur following a single administration of a test substance and provide information for estimating the median lethal dose (LD₅₀).

⁹ Sub-acute toxicity studies, on the other hand, involve repeated administration of a substance over a period of up to 28 days and are useful for evaluating potential effects on body weight, organ function, haematological and biochemical parameters, and tissue architecture.^{10, 11}

Given the widespread use of *Senna alata* in traditional medicine and the limited information available regarding its safety following repeated exposure, there is a need for further toxicological evaluation of the plant. Therefore, this study was designed to investigate the acute and subacute toxicity of *Senna alata* extracts in mice and Wistar rats. The findings of this study will contribute to the establishment of its safety profile and provide scientific evidence to support its rational therapeutic application.

Materials and Methods

Plant Collection, Authentication and Pulverisation of the Plant Leaves
Fresh leaves of *Senna alata* were collected in February, March and April 2024 from Kwara State Stadium, Ilorin, Nigeria. The plants were identified and authenticated in May 2024 by Bolu Ajayi at the herbarium unit of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Kwara State, where they were given the

*Corresponding author. mail: rtbaba@alhikmah.edu.ng
Tel: 07065391534

Citation: Baba RT, Salaudeen RA, Aladodo M, Dangana ZA, Muhammad ZK, Sowole HB, Sani HU, Yakubu F. Acute and Sub-acute Toxicity Studies of *Senna alata* on Mice and Wistar Rats. Trop J Phytochem Pharm Sci, 2026, 5(7); 663-671
<http://www.doi.org/10.26538/tjpps/v5i6.6>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

herbarium voucher number UILH/001/1059/2024. Leaves of *Senna alata* were washed thoroughly and air-dried: syphon after being in shade for two weeks. Dried leaves were ground into a fine powder using an industrial blender.

Preparation of Aqueous Extracts

For the preparation of the aqueous extract, 100 g of air-dried *Senna alata* leaves were finely pulverised and accurately weighed. The powdered material was transferred into sterile conical flasks, and 250 mL of distilled water was added to each sample. The containers were covered and maintained at room temperature ($25 \pm 2^\circ\text{C}$) for 72 hours. During this period, the mixtures were stirred intermittently to enhance the extraction of soluble bioactive compounds.

Following the 72-hour maceration period, the resulting mixtures were passed through Whatman No. 1 filter paper to separate the plant residues from the aqueous extract. The filtrates obtained were subsequently concentrated by lyophilisation (freeze-drying), which is suitable for minimising the degradation of heat-sensitive constituents and enhancing the stability and storage life of plant extracts.¹² Before freeze-drying, the filtrates were frozen at -20°C for 24 hours.

The pre-frozen samples were subsequently placed in a freeze-drying unit and dried under vacuum conditions, using a condenser temperature of approximately -50°C and a chamber pressure not exceeding 0.133 mBar. The lyophilisation process was maintained for 48 hours to facilitate the removal of water through sublimation until a completely dry, powdered extract was obtained. The dried extracts were carefully recovered, weighed to determine the extract yield, and transferred into airtight amber-coloured containers. The containers were stored at 4°C until required for the subsequent toxicological investigations.

Percentage yield of *Senna alata*

Formula;
$$\frac{\text{Weight of Extract (g)} \times 100}{\text{Weight of plant (g)}}^{13}$$

Preparation of Methanol Extracts

Methanolic extraction of the powdered *Senna alata* leaves was carried out using a Soxhlet extraction system. Approximately 100 g of the powdered plant material was measured and transferred into a porous extraction thimble. The loaded thimble was positioned inside the extraction chamber of the Soxhlet apparatus, which was fitted to a round-bottom flask containing 500 mL of methanol.

The solvent flask was placed on a heating mantle and heated under controlled conditions to facilitate continuous boiling of the methanol. As the solvent was heated, the resulting vapour passed upward into the condenser, where it cooled and returned as liquid to the extraction chamber containing the plant material. The condensed solvent was repeatedly passed through the powdered leaves and subsequently syphoned back into the flask. This extraction cycle was maintained for several hours and continued until the solvent returning through the syphon became clear, suggesting that the extraction of the soluble active constituents was essentially complete.

Upon completion of the Soxhlet extraction, the methanolic extract was separated from the solvent by concentration under reduced pressure. A rotary evaporator maintained at 50°C was used to remove the methanol and obtain the crude plant extract. The concentrated extract was transferred into sterile glass containers and preserved at 4°C until required for subsequent experimental procedures.¹³

Ethical clearance for the study

Ethical approval with reference number (UITH/CAT/189/21B/626) was obtained from the University of Ilorin Teaching Hospital prior to the commencement of the study.

Acute Toxicity Study

The acute toxicity experiment was conducted using 25 healthy adult mice, approximately 8 weeks old, with individual body weights

ranging from 30 to 35 g. The animals were randomly assigned to five groups (Groups 1–5), with five mice per group. Before commencement of the experiment, the animals were acclimatised to the laboratory environment for seven days and provided with standard feed and water ad libitum.

Following the acclimatisation period, the mice were fasted overnight prior to administration of the test substances. Groups 2–5 were orally administered aqueous and methanolic extracts of *Senna alata* leaves (AES and MES) at doses of 2000 and 3000 mg/kg body weight, respectively. Group 1 served as the control and received distilled water. All treatments were administered orally by gavage using an appropriate intubation cannula, with the administration volume standardised at 1 mL/100 g body weight (Table 1).

The animals were closely monitored for 48 hours following administration for any immediate signs of toxicity. Thereafter, observations continued for 14 days, during which physical and behavioural changes, including any visible signs of toxicity or mortality, were recorded.^{14, 15}

Subacute Toxicity Study

The subacute toxicity assessment involved 36 Wistar rats, which were randomly allocated into a control group and eight treatment groups, with four rats in each group. After randomisation, the animals were administered the aqueous and methanolic leaf extracts of *Senna alata* (AES and MES) orally for 28 consecutive days. The control animals received distilled water at a dose volume of 1 mL/kg body weight.

Treatment Groups 1 and 2 received AES and MES, respectively, at 500 mg/kg body weight. Groups 3 and 4 were administered the corresponding extracts at 1000 mg/kg, while Groups 5 and 6 received 2000 mg/kg. Groups 7 and 8 were treated with the respective extracts at 3000 mg/kg body weight (Table 2). Throughout the 28-day experimental period, the body weights of the animals, together with their food and water consumption, were monitored and recorded weekly.

At the completion of the treatment period, all animals were sacrificed for sample collection and further investigations. The rats were rendered unconscious by inhalation of chloroform using cotton wool saturated with 3.5% chloroform. Blood samples were subsequently obtained through cardiac puncture using a 5-mL syringe fitted with an appropriate needle. The collected blood was dispensed into two types of containers: EDTA-containing bottles for haematological investigations and plain bottles for biochemical analyses.

Blood samples collected into the plain tubes were allowed to stand at room temperature for approximately 15 minutes to facilitate clot formation and serum separation. The samples were then centrifuged at 3,600 rpm for 15 minutes. The resulting sera were carefully separated and stored at -20°C until biochemical analyses were performed.^{14, 15}

Haematological parameters were determined using a DYMINDD DF51 haematology analyser, whereas biochemical parameters were assessed using a BIOELAB ES-105 analyser. Histopathological evaluation was performed on liver and kidney tissues obtained from rats exposed to the highest doses of the plant extracts (2000 and 3000 mg/kg body weight). The tissues were processed using standard paraffin-wax tissue-processing techniques for subsequent microscopic examination.^{15, 16}

Preparation of Tissue Sections

At the end of the four-week experimental period, the animals were euthanised, and the liver and kidney tissues were carefully excised. The freshly collected tissues were immediately immersed in 10% formal saline and fixed for 48 hours. Representative portions were subsequently trimmed into thin sections, placed in appropriately labelled tissue cassettes, and subjected to routine paraffin-wax tissue processing.

The tissue specimens underwent dehydration through a graded series of alcohol concentrations (70%, 80%, 90%, and absolute alcohol), with each grade maintained for approximately 2 hours. Following dehydration, the tissues were left overnight in absolute alcohol. Clearing was then performed using two successive changes of xylene, each lasting 1 hour. This was followed by infiltration and

impregnation with two changes of molten paraffin wax, with each change maintained for 1 hour.

After processing, the tissues were embedded in molten paraffin wax and rapidly cooled on ice to form solid tissue blocks. Serial ribbon sections, approximately 3 μm thick, were obtained from the paraffin blocks using a LEICA RM 2135RT rotary microtome. The tissue ribbons were transferred onto a floating bath containing 20% alcohol as the floating fluid and subsequently picked up onto clean glass slides. Each slide was properly identified using the corresponding tissue-cassette number. The prepared slides were placed in a hot-air oven maintained at 65°C to facilitate adequate adhesion of the tissue sections to the glass surface.¹⁷

Histological Staining

Haematoxylin and Eosin (H&E) Staining

Haematoxylin was used as the primary nuclear stain, while eosin served as the counterstain. Prior to staining, the paraffin-embedded tissue sections were deparaffinised in xylene for 1 hour and subsequently rehydrated through descending concentrations of alcohol, from absolute alcohol to 70% alcohol. The sections were rinsed with water before being immersed in haematoxylin for 15 minutes.

Following haematoxylin staining, the sections were washed with water and briefly differentiated using 1% acid alcohol. The slides were then placed under running tap water for approximately 10 minutes to achieve bluing. Counterstaining was subsequently carried out with eosin for 2 minutes. The stained sections were dehydrated through ascending concentrations of alcohol, progressing from 70% alcohol to absolute alcohol, cleared in xylene, and finally mounted with Dibutyl Phthalate Xylene (DPX) for microscopic examination.¹⁷

Photomicrography

The prepared slides were initially examined microscopically using the $\times 10$ objective lens to assess the general tissue architecture. Sections showing pathological or notable histological changes were further examined under the $\times 40$ objective lens to obtain a more detailed view of the lesions. Photomicrographs were captured at both magnifications where appropriate for documentation and subsequent interpretation.

Haematological Analysis

Haematological parameters of the Wistar rats were determined using a DYMIND DF51 automated haematology analyser according to the manufacturer's operating instructions. Prior to sample analysis, the instrument was appropriately calibrated, and routine quality-control procedures were performed using standard reference control materials to ensure reliable analytical performance.

Approximately 1 mL of well-mixed whole blood collected from each animal was introduced into the analyser for automated processing. The instrument generated the complete blood count (CBC), including red blood cell (RBC) count, haemoglobin concentration, packed cell volume (PCV), white blood cell (WBC) count, and platelet count. The analytical results were displayed and printed from the instrument and subsequently recorded for statistical analysis.^{14, 15}

Biochemical Parameters

Serum Creatinine

Serum creatinine concentration was determined using Agappe creatinine reagent kits with a BIOELAB ES-105 automated chemistry analyser, following the manufacturer's recommended procedure. Following blood collection and centrifugation, the resulting serum was carefully separated into appropriately labelled tubes for analysis. Prior to testing, the analyser was calibrated using Agappe calibrators, and assay performance was assessed with low- and high-level control sera.

Creatinine was measured based on the kinetic alkaline picrate (Jaffe) method. In this reaction, creatinine interacts with picric acid in an alkaline medium to produce a coloured complex, the formation of which is proportional to the creatinine concentration. For the assay, 1000 μL of reagent was combined with 100 μL of serum sample,

mixed gently, and introduced into the analyser. The instrument carried out the required reagent and sample handling and measured the rate of colour development photometrically at 505 nm. Creatinine concentrations were automatically calculated from the established calibration curve. The generated results were stored electronically, and only values associated with acceptable quality-control results were included in the final analysis.¹⁸

Serum Urea

Serum urea concentration was evaluated using Precision urea reagent kits on the BIOELAB ES-105 automated chemistry analyser according to the manufacturer's instructions and the analyser's programmed assay protocol. Before commencement of analysis, the relevant reagent lot information was entered into the system, and calibration was performed using the Precision calibrator. Assay reliability was subsequently assessed with both low- and high-concentration control sera.

The analysis employed the enzymatic urease-Berthelot method. During the assay, the analyser automatically aspirated the specified quantities of serum and reagent and performed the required incubation and reaction timing. The resulting change in optical density was measured at 540 nm, as specified by the reagent manufacturer. Urea concentration was automatically derived by the analyser using the calibration curve. Results were electronically recorded, and only measurements obtained when the quality-control values were within the specified acceptable limits were regarded as valid.¹⁸

Alanine Aminotransferase (ALT)

Serum ALT activity was assayed using Agappe ALT reagent kits with the BIOELAB ES-105 automated chemistry analyser in accordance with the manufacturer's protocol. The system was calibrated with Agappe calibrators, while assay performance was monitored using normal and pathological control sera.

The assay was based on the IFCC-recommended method without pyridoxal phosphate. In this reaction, ALT catalyses the transfer of the amino group from alanine to 2-oxoglutarate, producing pyruvate and glutamate. The pyruvate formed is subsequently converted to lactate by lactate dehydrogenase, during which NADH is oxidised to NAD^+ . For the determination, 1000 μL of reagent was mixed with 100 μL of serum and gently homogenised before being aspirated into the analyser. Enzyme activity was determined from the decrease in absorbance of NADH at 340 nm and reported in U/L. Only results associated with satisfactory quality-control performance were accepted for analysis.¹⁸

Aspartate Aminotransferase (AST)

Serum AST activity was measured using Agappe AST reagent kits on the BIOELAB ES-105 automated chemistry analyser, following the manufacturer's recommended procedure. The separated serum samples were transferred into properly labelled tubes before analysis. Calibration of the analyser was performed using Agappe calibrators, and analytical performance was monitored using low- and high-level control materials.

The assay utilised the IFCC method without pyridoxal phosphate. AST catalyses the transfer of an amino group from aspartate to 2-oxoglutarate, resulting in the formation of oxaloacetate and glutamate. Oxaloacetate is subsequently converted to malate through the action of malate dehydrogenase, with simultaneous oxidation of NADH to NAD^+ . The reduction in NADH absorbance was monitored at 340 nm. A volume of 1000 μL of reagent was combined with 100 μL of serum, mixed gently, and aspirated into the analyser. AST activity was automatically calculated by the instrument and expressed in U/L. Results were considered acceptable only when the corresponding quality-control measurements were within the specified limits.¹⁸

Alkaline Phosphatase (ALP)

Serum ALP activity was determined using Agappe ALP reagent kits with the BIOELAB ES-105 automated chemistry analyser according to the manufacturer's instructions. The analyser was calibrated using

Agappe calibrators, and assay validity was assessed using low- and high-level control sera.

The analytical procedure was based on the IFCC p-nitrophenyl phosphate (pNPP) method. Under alkaline conditions, ALP hydrolyses pNPP to produce p-nitrophenol, which develops a yellow colouration. The analyser monitored the increase in absorbance resulting from the reaction at 405 nm and calculated ALP activity in U/L. For the assay, 1000 µL of reagent was combined with 20 µL of serum sample, gently mixed, and aspirated into the analyser. Only results obtained with quality-control values falling within the manufacturer's acceptable range were considered valid for subsequent analysis.¹⁸

Total Protein

Serum total protein concentration was quantified using Agappe Total Protein reagent kits on the BIOELAB ES-105 automated chemistry analyser in accordance with the manufacturer's instructions. Following centrifugation, the separated serum was transferred into appropriately labelled tubes. The analyser was calibrated with Agappe calibrators, and assay performance was verified using low- and high-level control sera.

Protein concentration was determined using the Biuret method, in which peptide bonds in proteins react with copper ions under alkaline conditions to form a violet-coloured complex. The intensity of the resulting colour, which is proportional to the protein concentration, was measured spectrophotometrically at 546 nm. The analyser subsequently calculated the serum total protein concentration in g/dL using the calibration curve. Only measurements that satisfied the

established internal quality-control criteria were retained for statistical analysis.¹⁸

Statistical Analysis

Data were statistically analysed using SPSS version 25. The p-values were determined using the t-test, and a significance level of $p < 0.05$ was considered statistically significant.

Results and Discussion

The extraction process produced different yields depending on the solvent employed. The methanol extract of *Senna alata* gave the greatest extraction yield of 7.9%, whereas the aqueous extract produced a lower yield (Table 3). The difference may be related to the physicochemical properties of the extraction solvents. Methanol has an intermediate polarity and can dissolve a relatively broad spectrum of plant constituents, including compounds with polar and moderately non-polar characteristics. Water, on the other hand, preferentially extracts highly polar substances. The greater yield obtained with methanol is in agreement with earlier reports indicating that the polarity of the extraction solvent substantially influences the quantity and diversity of phytochemicals recovered from medicinal plants. Methanol has also been reported to exhibit greater extraction efficiency than water because of its wider solubilising ability and capacity to penetrate tissues.^{19, 20}

Table 1: Protocol table for acute toxicity study of *Euphorbia hirta*

Groups	Number of mice	Extract type	Dose mg/kg	Volume administered (ml/bw)
1	5	Water	-	1 ml/100g
2	5	AES	2000	1 ml/100g
3	5	MES	2000	1 ml/100g
4	5	AES	3000	1 ml/100g
5	5	MES	3000	1 ml/100mg

Key: AES: aqueous extract of *Senna alata*, MES: methanol extract of *Senna alata*

Table 2: Protocol table for subacute toxicity

Group	Number of rats	Extracts	Dose(mg/kg)	Remarks
Control	4	Distilled water	0	-
1	4	AES	500	Low
2	4	MES	500	Low
3	4	AES	1000	Medium
4	4	MES	1000	Medium
5	4	AES	2000	High
6	4	MES	2000	High
7	4	AES	3000	Very high
8	4	MES	3000	Very high

Key: AES: aqueous extract of *Senna alata*, MES: methanol extract of *Senna alata*

Table 3: Percentage yield of *Senna alata* extracts

Plants	Solvents	Weight of plants/g	Weight of extracts/g	Percentage yield %
<i>Senna alata</i>	Aqueous	100	6.2	6.2
	methanol	100	7.9	7.9

The acute toxicity investigation indicated that both aqueous and methanol extracts of *S. alata* were well tolerated by the experimental animals. Administration of single oral doses of 2000 and 3000 mg/kg did not result in mortality or observable behavioural abnormalities. Since no deaths occurred at the maximum dose administered, the estimated median lethal dose (LD₅₀) was greater than 3000 mg/kg. This finding suggests that the extracts have relatively low acute toxicity at the doses investigated. However, because doses above 3000 mg/kg were not evaluated, the result is appropriately expressed as LD₅₀ > 3000 mg/kg rather than as an exact LD₅₀ value. The observation is comparable with findings from toxicological investigations of several medicinal plants in which high oral LD₅₀ values have been associated with low acute toxicity.^{21, 22} The 28-day repeated-dose study also showed good tolerance of the extracts. Animals administered 500, 1000, 2000, and 3000 mg/kg/day

continued to gain body weight during the experimental period, and the changes did not differ significantly from those recorded in the control animals (Fig. 1). Relative organ weights were likewise not significantly altered. The maintenance of normal body weight throughout the treatment period suggests that the extracts did not substantially interfere with nutritional status, metabolic processes, or normal growth. Likewise, the absence of significant changes in organ weights provides no indication of treatment-related organ enlargement or atrophy (Fig. 2). Since considerable reductions in body or organ weight may accompany systemic toxicity, the present observations support the relative safety of *S. alata* during the period examined. Similar findings have been documented in previous studies in which repeated administration of medicinal plant preparations produced no meaningful changes in body weight or general behaviour.^{23, 24}

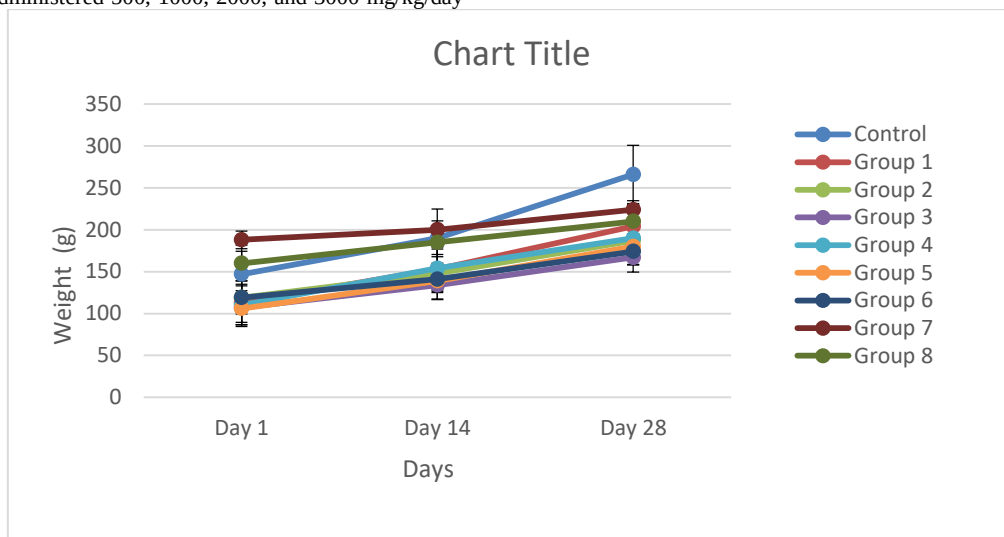


Fig 1: Graph showing the weight of Wistar rats before and after exposure to the extracts. Values are expressed as mean of six replications $\pm$ standard error of the mean (SEM)

Assessment of serum biochemical parameters showed that administration of *S. alata* produced little effect on most of the parameters examined (Fig. 3). Animals receiving the lower and intermediate doses showed no significant differences in ALP, creatinine, total protein, urea, and other measured indices compared with the control group. These results indicate that renal function and major aspects of hepatic function were generally maintained during the treatment period. Biochemical measurements such as creatinine and urea are useful indicators of renal function, whereas ALT, AST, and ALP are commonly employed in evaluating possible hepatic injury or dysfunction.^{25, 26} Despite the general stability of the biochemical parameters, slight increases in ALT and AST were detected in animals exposed to the

higher doses, particularly at 2000 and 3000 mg/kg (Fig. 3). The changes were more evident at the highest dose and may reflect mild hepatocellular stress resulting from increased exposure to the extract. Nevertheless, the lack of substantial alterations in ALP, total protein, and other biochemical indices suggests that the enzyme elevations were not associated with severe hepatic dysfunction. Similar increases in liver enzymes have been described following exposure to high concentrations of certain medicinal plant preparations.²⁶ The biochemical pattern observed in the present study therefore appears more consistent with a mild, dose-related hepatic response than with pronounced hepatotoxicity.

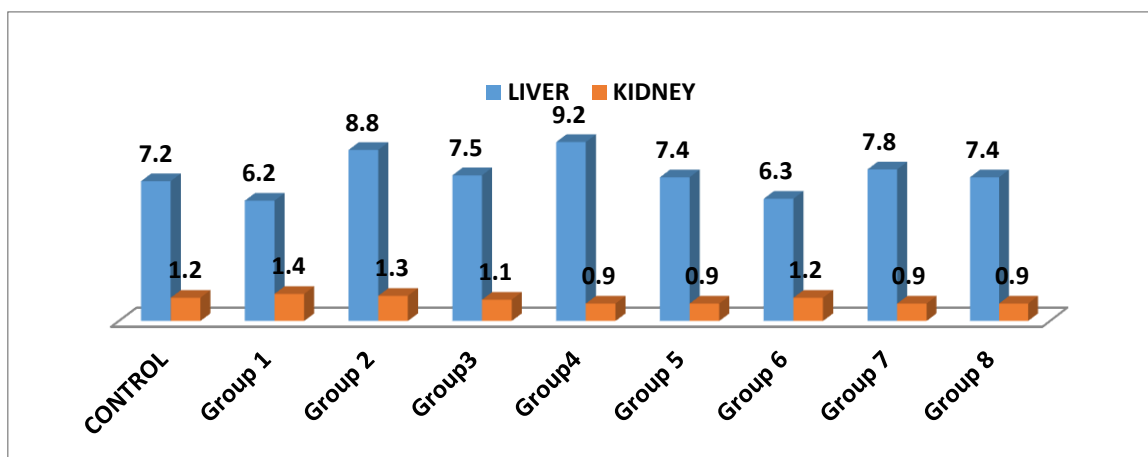


Fig 2: Bar chart showing the weight of the organs of the Wistar rats.

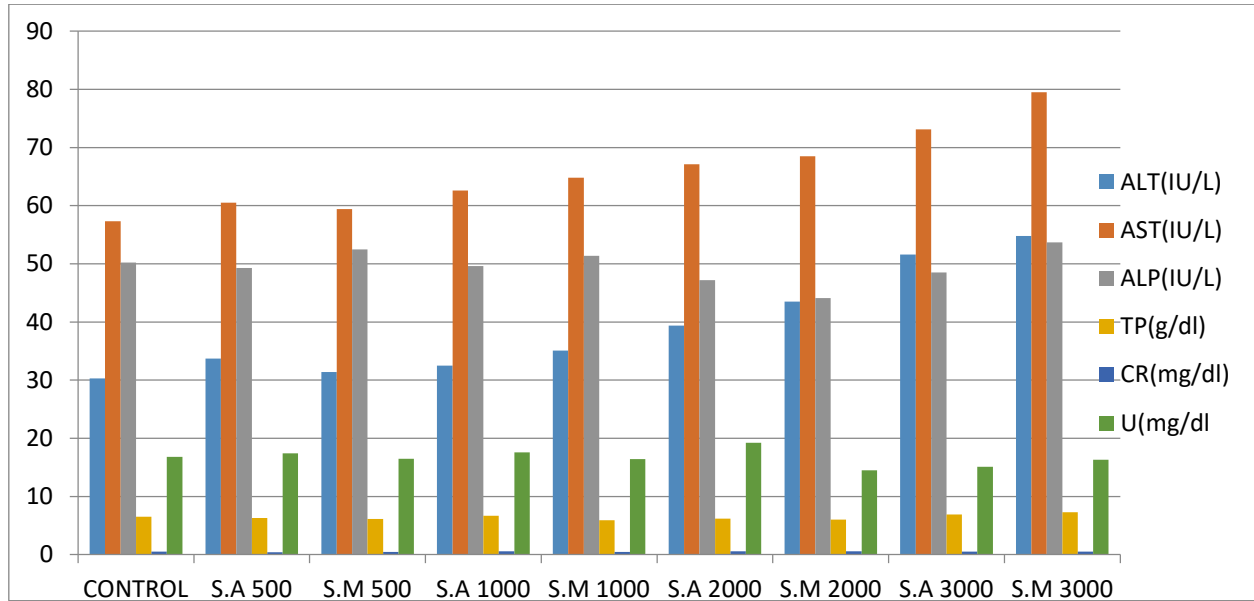


Fig 3: Biochemical parameters of *Senna alata* aqueous and methanol extracts on Wistar rats (ALT and AST for 2000mg/kg and 3000mg/kg; mean significance at p-value < 0.05 when compared with control)

KEY:

S.A - *Senna alata* aqueous
S.M- *Senna alata* methanol
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
TP: Total Protein
CR: Creatinine
U: Urea

The haematological results revealed that most blood parameters remained comparable between the treated and control groups (Fig. 4). In particular, there were no indications of anaemia, leukopenia, or thrombocytopenia following administration of the extracts. This suggests that treatment did not produce appreciable suppression of blood-cell production or adverse effects on the haematopoietic system. The finding agrees with previous investigations in which

administration of medicinal plant extracts caused no significant disruption of haematological parameters.²⁷ An important exception in the present study was the significant increase in platelet counts observed at the higher doses. This elevation may be related to a mild physiological or inflammatory response, particularly because inflammatory changes were also observed in the liver tissues of animals exposed to the higher concentrations.

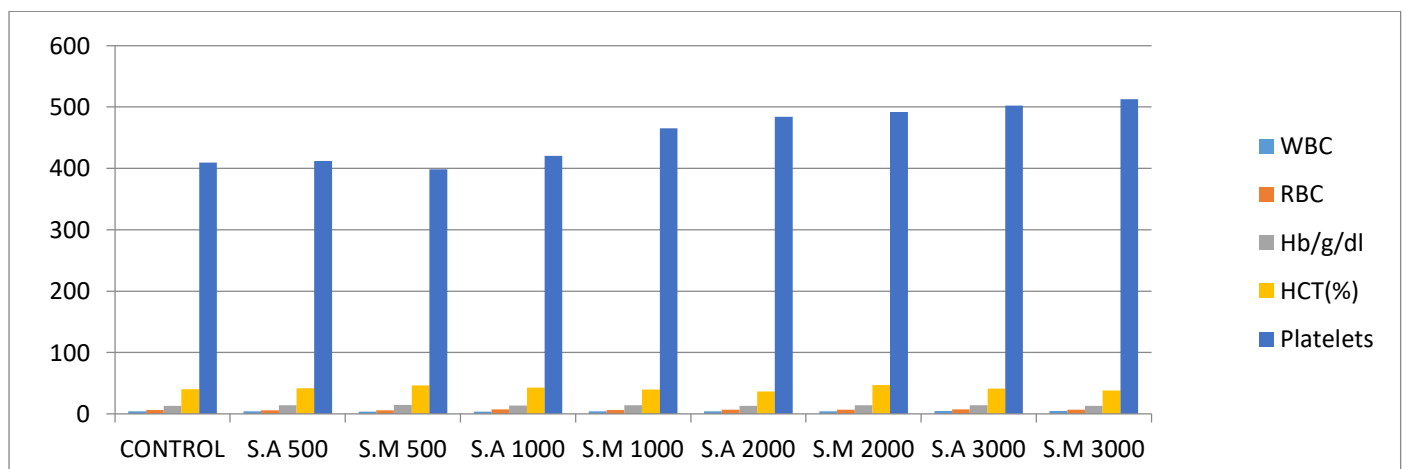


Fig 4: Haematological parameters of *Senna alata* aqueous and methanol extracts on Wistar rats (Platelet: mean significance at p-value < 0.05 when compared with control)

Microscopic assessment of the kidneys showed that renal structures were largely preserved in all experimental groups (Figs. 5–9). The

glomeruli and renal tubules maintained their normal appearance, while the interstitial regions showed no significant pathological changes. No

evidence of marked acute or chronic renal injury was identified. These histological findings are consistent with the biochemical observations, particularly the absence of significant changes in serum creatinine and

urea, and further suggest that the extracts did not produce appreciable renal damage at the doses used.

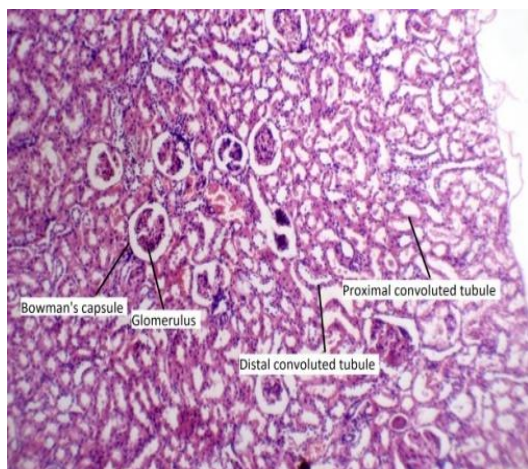


Fig 5: Control kidney

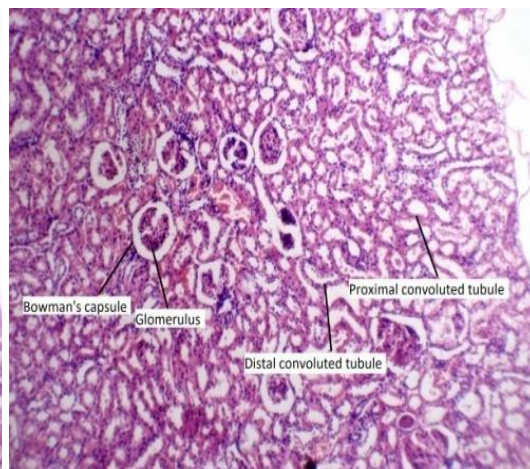


Fig 6: Group 5 kidney

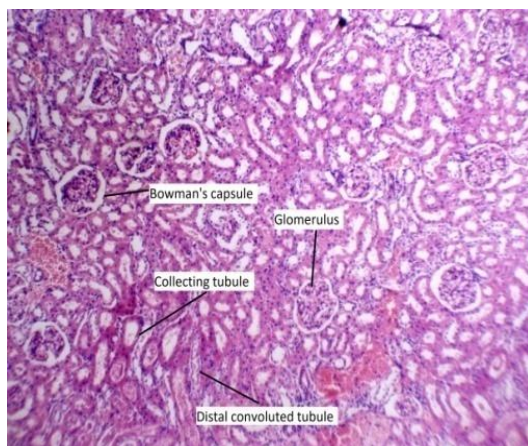


Fig 7: Group 6 kidney

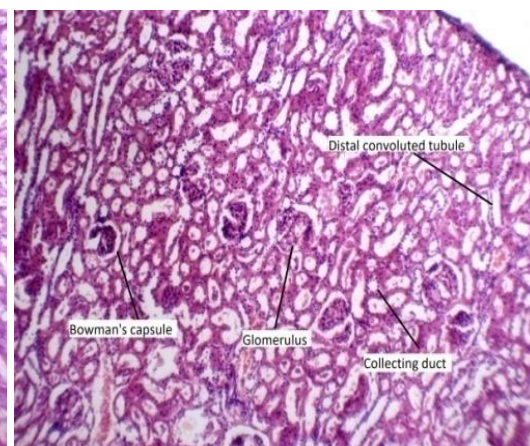


Fig 8: Group 7 kidney

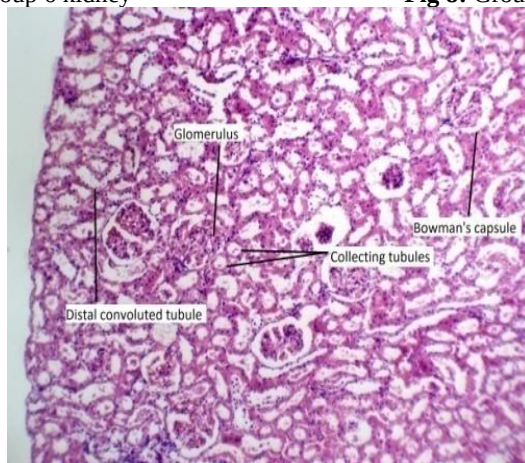


Fig 9: Group 8 kidney

Examination of the liver sections demonstrated generally preserved hepatic architecture in the treated animals (Figs. 10–14). However, some changes became apparent in animals exposed to the higher doses. These included mild widening of the hepatic sinusoids and slight inflammatory changes around the portal tracts, although the arrangement of the hepatocyte cords remained largely intact. Coarse granular material was also observed within the cytoplasm of some hepatocytes. In one of the high-dose groups, more distinct changes were identified, including marked sinusoidal dilation, the presence of

haemosiderin-containing Kupffer cells, and moderate portal tract inflammation. The occurrence of these changes at higher exposure levels suggests that large doses of the extract may impose some degree of hepatic stress.

The histological observations correspond with the mild elevations in ALT and AST recorded in the biochemical assessment. This agreement between biochemical and microscopic findings suggests that the hepatic changes were treatment-related and may have been influenced by dose. Nevertheless, the alterations were relatively

limited, and there was no evidence of extensive hepatocellular necrosis, severe degeneration, or chronic liver damage in the treated groups. Previous studies have similarly reported minor hepatic

alterations following prolonged or high-dose administration of medicinal plant extracts without progression to severe hepatotoxicity.^{27, 28}

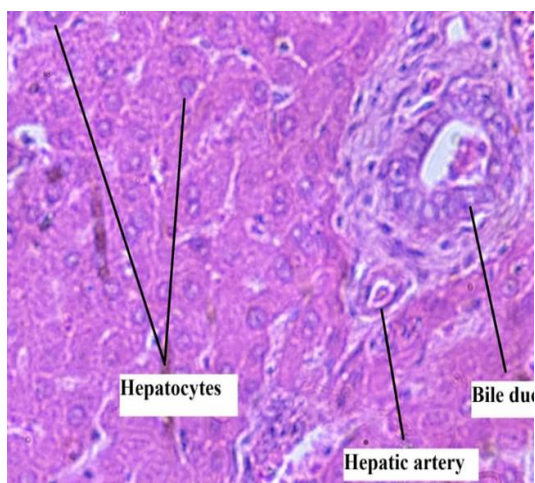


Fig 10: Control liver

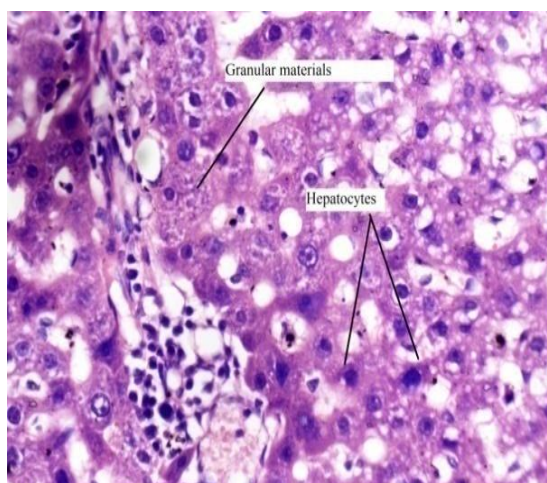


Fig 11: Group 5 Liver

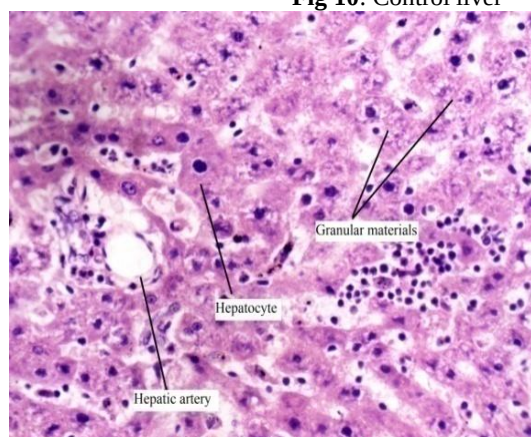


Fig 12: Group 6 Liver

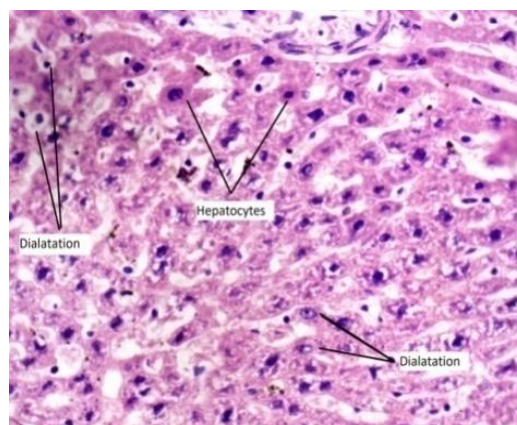


Fig 13: Group 7 Liver

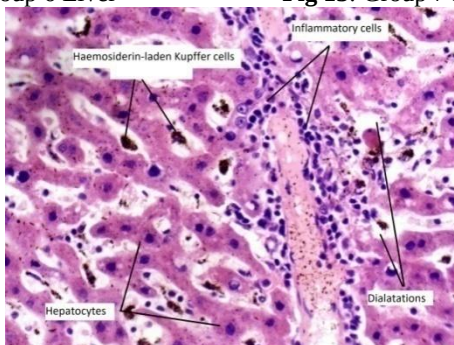


Fig 14: Group 8 Liver

Taken together, the findings indicate that the aqueous and methanol leaf extracts of *S. alata* have a relatively favourable safety profile at the doses investigated. The absence of mortality or abnormal behaviour during acute exposure, together with the normal progression of body weight, stable relative organ weights, largely unchanged haematological indices, and minimal alterations in most biochemical parameters during the subacute study agree with the overall safety of the extracts. However, the increases in ALT and AST, elevated platelet counts, and mild hepatic microscopic changes observed particularly at the higher doses indicate that excessive exposure may place some stress on the liver. Consequently, although the extracts appear well tolerated at the investigated doses, caution may be necessary when considering very high doses or prolonged administration. Further investigations involving longer treatment

periods, additional hepatic biomarkers, and oxidative stress parameters would be useful for establishing the long-term safety profile of *S. alata*.

Conclusion

The toxicological profile of *Senna alata* extracts indicates that these extracts are safe at tested doses, although higher concentrations may warrant caution due to mild histological and biochemical changes.

Future Prospects

Further studies should evaluate the long-term and chronic toxicity of *Senna alata*, particularly at higher doses. Additional investigations of oxidative stress, inflammatory markers, and hepatic and renal biomarkers are recommended to clarify the mechanisms underlying

the mild changes observed and establish the long-term safety of the extracts.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

- Latif R, Nawaz T. Medicinal plants and human health: a comprehensive review of bioactive compounds, therapeutic effects, and applications. *Phytochem Rev.* 2025; 24:1-44.
- World Health Organization. WHO traditional medicine strategy 2014-2023. Geneva: World Health Organization; 2013.
- Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4:177. doi:10.3389/fphar.2013.00177.
- Abille JMN. A review on the medicinal plant *Cassia alata* (L.) Roxb. (Fabaceae). Dasmariñas (Philippines): Biological Sciences Department, College of Science and Computer Studies, De La Salle University-Dasmariñas; 2015. p. 1-10.
- Oladeji OS, Adelowo FE, Ayodele DT, Odelade KA. Ethnobotanical description and biological activities of *Senna alata*. *Evid Based Complement Alternat Med.* 2020;2020:2580259. doi:10.1155/2020/2580259.
- Karthika K, Jamuna S, Paulsamy S. Chemical constituents, traditional uses and pharmacological activities of *Cassia alata* (*Senna alata*): a review. *Int J Sci Res Rev.* 2020;9(2):1-15.
- Fatmawati S, Shimizu K, Kondo R. Chemical constituents, usage and pharmacological activity of *Senna alata*. *J Nat Med.* 2020; 74(1):1-13. doi:10.1007/s11418-019-01352-2.
- Afolayan AJ, Wintola OA. A review of medicinal plants with hepatoprotective activities. *J Med Plants Res.* 2014; 8(5):167-178.
- Organisation for Economic Co-operation and Development. OECD guideline for testing of chemicals 423: acute oral toxicity—acute toxic class method. Paris: OECD; 2001.
- Organisation for Economic Co-operation and Development. OECD guideline for testing of chemicals 407: repeated dose 28-day oral toxicity study in rodents. Paris: OECD; 2008.
- Adeoye AO, Ake AS. Safety assessment of garlic (*Allium sativum*) extracts on body weight and haemato-biochemical parameters in male Wistar rats. *Trop J Phytochem Pharm Sci.* 2026; 5(3):518-522. doi:10.26538/tjpps/v5i3.5.
- Abubakar A, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. *J Pharm Bioallied Sci.* 2020; 12(1):1-10.
- Manandhar S, Al-Saadi AA, Al-Faifi ZE. Searching for natural herbicides in methanol extracts of eight plant species. *Bull Natl Res Cent.* 2019; 43(1):1-7.
- Roy S. Acute and sub-acute toxicity studies on the effect of *Senna alata* in Swiss albino mice. *Int J Pharm Sci Res.* 2016; 7(6):2505-2510. doi:10.13040/IJPSR.0975-8232.7(6).2505-10.
- Srivastava AK, Kumar A, Kumar D. Biological screening, toxicity, and regulations of herbal drugs. In: *The essentials of herbal drugs*. Oakville: Apple Academic Press; 2026; p. 251-279.
- Owolarafe T, Ihegboro G, Salawu K, Ononamadu C, Fadilu M, Musa B. Toxicological investigation of aqueous extract of *Ziziphus mauritiana* leaves on wistar rats. *International Journal of Traditional and Complementary Medicine Research.* 2022; 8; 3(2):91-100.
- Muhammad A, Adamu M, Bello A. Histological processing and tissue sectioning for microscopic analysis. *J Histol Lab Tech.* 2022; 45(3):210-217.
- Amoateng EO, Amoateng P, Ossei PP, Fenteng EA, Amponsah IK, Ayibor WG, Adjei S, Narh-Bedu T. An acute and sub-acute toxicological assessment of *Reissantiaindica* plant extract in male Sprague-Dawley rats: Hematological, serum biochemical and histopathology. *Sci Afr.* 2024. 1;23:e02089.
- Kumari K, Adhikari P, Pandey A, Samant SS, Lal M, Pande V. Influence of solvent polarity on phytochemicals, antioxidants, and antimicrobial properties of *Delphinium denudatum*: A medicinal herb from Sainj Valley, Himachal Pradesh, India. *Bioact.* 2024; 2(1):30-40.
- Tarek K, Farid A, Safwat G. Extraction of grape seeds by different solvents affects the activities of the resultant extract. *AMB Express.* 2025; 15(1):1-14.
- Corvaro M, Gehen S, Andrews K, Chatfield R, Arasti C, Mehta J. GHS additivity formula: a true replacement method for acute systemic toxicity testing of agrochemical formulations. *Regul Toxicol Pharmacol.* 2016; 82:99-110.
- Sharma R. A comprehensive overview of toxicity in isolated compound and in herbal plant. *World J Pharm Res.* 2015; 14(10):659-691.
- Hasan MZ, Rahman MM, Islam MS. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. *Biomed Res Int.* 2013; 81-121.
- Zhang L, Xu M, Guan Q, Jiang J, Sun K, Manirafasha E. Determination of vegetable tannins from plants in China. *Biofuels Bioprod Biorefin.* 2023; 17(3):592-601.
- Musa I. Liver and kidney functional indices of pregnant rats following the administration of the crude alkaloids from *Senna alata* (Linn. Roxb.) leaves. *Iran J Toxicol.* 2012; 6:16-23.
- Izunya AM, Akpamu U, Uzzi HO, Oaikheni GA, Ujaddughe OM. Effects of ethanolic extract of *Euphorbia hirta* leaf on biochemical parameters and histology of liver and kidney in rats. *FUOYE J Biomed Res.* 2024; 1(2):148-158.
- Ping W, Bi K, Lin Y. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. *Biomed Res Int.* 2013; 182-264.
- Khalaf RR, Abouzeinab NS, Khalil MI. Effects of Lebanese folk herbs on adult male rats: hepatic and renal toxicity, histological, and biochemical studies. *Nutrients.* 2025; 17(5):875.