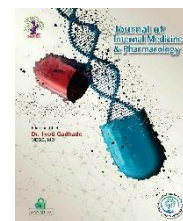




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Research Article

Toxicological Assessment of *Murraya Koenigii* Fruit Extract: Acute and Subacute Evaluation in experimental animals**Atish Waghmare¹, Yeshpratap Singh Rana², Jagdish Manwar¹**^{1,3}Kamalprakash Pharmacy College and Research Centre, Karanja. India²School of Pharmacy, Glocal University, Saharanpur (U.P) India

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ABSTRACT

The current investigation was aimed to assess anti-anaphylactic, and anti-asthmatic activity of aqueous extract of *psidium guajava* (Myrtaceae) on experimental animals. Anaphylaxis was induced by administration of horse serum subcutaneously in albino Wistar rats. Extracts of *psidium guajava* were administered to the rats in dose of 125,250 and 350 mg/kg b.w. orally for 14 days. At the end of treatment, we assessed the modulatory effect on experimentally induced airway inflammation in rats. The effect of *psidium guajava* on oxidative stress, TNF- α and lung histopathology. Anti-asthmatic activity of extracts of *psidium guajava* was also studied on Milk induced eosinophilia and leukocyte count model. The treatment with extracts of *psidium guajava* produced significant decrease in eosinophils and leukocyte count. Thus, these findings concluded that *psidium guajava* could be effectively used in the treatment of anaphylaxis and asthma.

Keywords: anti-anaphylactic activity; anti-asthmatic activity; *Psidium guajava*; oxidative stress; TNF- α **** Corresponding author****Atish Waghmare^{1*}**²School of Pharmacy, Glocal University, Saharanpur (U.P.), IndiaE-mail addresses: atishnamdeo@gmail.com

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1.Introduction

Medicinal plants have played a significant role in traditional healthcare systems for centuries, particularly in regions such as Asia and Africa, where modern pharmaceuticals may be inaccessible to a large portion of the population. One such plant is *Murraya koenigii*, commonly known as the curry leaf, which has been widely used in folk medicine for its therapeutic properties. The plant is well known in Ayurvedic and Unani systems of medicine for its efficacy in treating a variety of ailments including diabetes, inflammation, dysentery, and liver disorders. *Murraya koenigii* belongs to the Rutaceae family and is indigenous to the Indian subcontinent but is also cultivated in other tropical and subtropical regions worldwide [1,2].

The bioactive compounds present in *Murraya koenigii* have drawn scientific interest due to their pharmacological potential. Studies have shown that the plant contains a wide array of biologically active components such as alkaloids, flavonoids, tannins, and glycosides. These compounds are known to possess antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties, among others. Despite its well-documented therapeutic uses, there is a paucity of scientific data on the safety of long-term or high-dose consumption of its fruit extract, which is often used in traditional formulations. The safety assessment of medicinal plants, particularly those intended for human consumption, is critical to understanding their potential toxicological effects and establishing safe dosage levels for therapeutic use [3,4].

Murraya koenigii has gained increasing attention in recent years for its potential role in managing metabolic disorders like diabetes, obesity, and hyperlipidemia. However, while its pharmacological benefits have been studied to some

extent, there is a growing need for comprehensive toxicological evaluations. Toxicity studies, especially acute and subacute studies, are fundamental in identifying any potential harmful effects following single or repeated exposure to medicinal plant extracts. Acute toxicity studies typically evaluate the immediate effects of a single large dose, identifying the lethal dose (LD50) and the range of non-lethal doses, while subacute toxicity studies assess the impact of repeated exposure over a period (typically 28 days) to observe any cumulative effects [5,6].

The importance of toxicological evaluations cannot be overstated, particularly given the increasing use of herbal medicines worldwide. The use of natural plant products in pharmaceutical preparations is becoming more popular, but it is crucial that these products undergo rigorous safety evaluations. The potential for adverse effects, including those related to long-term use or high-dose consumption, must be thoroughly explored. Subacute toxicity studies are particularly valuable as they can reveal cumulative effects on various organ systems, changes in physiological parameters, and potential histopathological changes that may not be evident from acute toxicity studies alone [7].

In this context, the present study was undertaken to evaluate both the acute and subacute toxicity of the ethanolic fruit extract of *Murraya koenigii* in experimental animals. The choice of an ethanolic extract is significant, as ethanol is a commonly used solvent in traditional extraction methods and effectively isolates a broad spectrum of bioactive compounds. This study aimed to assess the safety profile of the fruit extract, focusing on physiological, biochemical, and histopathological parameters in both mice and rats. By investigating the effects of the extract in both short-term (14-day acute study) and medium-term (28-day subacute

study) exposure models, this research provides a comprehensive understanding of the safety and potential risks associated with the use of *Murraya koenigii* fruit extract [8].

In the acute toxicity study, different doses of the ethanolic fruit extract were administered to mice, and the animals were observed for any signs of toxicity, mortality, and behavioral or physiological changes over a period of 14 days. This part of the study was designed to determine the extract's LD50, or the dose at which 50% of the test animals succumb to the effects of the substance. The absence of any significant adverse effects would indicate the potential safety of the extract for human consumption at moderate doses [9].

The subacute toxicity study, on the other hand, involved the repeated oral administration of the extract over 28 days in rats, at different dosage levels. This part of the study aimed to assess the cumulative effects of the extract on the animals' physiological and biochemical parameters, such as body weight, food consumption, organ weights, and blood chemistry, including liver and kidney function markers. Furthermore, histopathological examinations were conducted to observe any potential damage to vital organs such as the liver, kidneys, heart, lungs, and brain. Histopathology is a critical aspect of toxicology, as it provides direct evidence of tissue damage or alterations at the cellular level, which may not always be reflected in biochemical or physiological parameters [10].

Given the wide use of *Murraya koenigii* in traditional medicine, particularly in formulations aimed at managing chronic conditions like diabetes and hyperlipidemia, it is essential to establish a comprehensive safety profile for this plant. This study fills a crucial gap in the literature by providing detailed toxicological data on the fruit extract of

Murraya koenigii, which can guide its safe therapeutic use and support further research into its pharmacological properties.

The results of this study will help establish safe dosage levels for *Murraya koenigii* fruit extract and provide a foundation for future studies on its long-term safety and efficacy. Additionally, this research highlights the need for rigorous toxicological evaluations of medicinal plants that are commonly used in traditional remedies, to ensure their safe integration into modern healthcare practices [11].

2.. Material and Method

2.1 Procurement and Authentication of Drug

Fruits of *Murraya koenigii* were collected from the region of Yavatmal district Maharashtra, India during the month of June to September 2012. The fruits were authenticated by Dr. N. M. Dongarwar, Head of the Department; Botany Department, RTM Nagpur University, Nagpur. A voucher specimen (No. 9916) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur, India [12].

2.2 Experimental Animals

Swiss albino mice, weighing 25–30 g, were used for the acute toxicity study, while Wistar rats, weighing 100–150 g, of both sexes, were selected for the subacute toxicity study. All animals were procured from [name the animal supplier or facility] and were housed under standard laboratory conditions with a 12-hour light/dark cycle at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of 55–60%. Animals were provided with a standard laboratory diet and water ad libitum throughout the experiment. The animals were acclimatized to laboratory conditions for seven days before the commencement of the study [13].

2.3 Acute Toxicity Study (14 Days)

The acute oral toxicity study was performed according to the guidelines set forth by the Organization for Economic Cooperation and Development (OECD) 423. A total of four groups of Swiss albino mice ($n = 5$ per group) were used in this study. Group 1 served as the control and received distilled water, while groups 2, 3, and 4 received the ethanolic extract of *Murraya koenigii* fruits at doses of 300, 500, and 900 mg/kg body weight, respectively. The extract was administered orally using an intragastric tube. After administration, the animals were observed individually for signs of toxicity such as changes in skin, fur, eyes, and mucous membranes, as well as changes in behavior, tremors, convulsions, salivation, diarrhea, lethargy, and mortality. These observations were made at 30 minutes, 4 hours, 24 hours, and daily thereafter for 14 days. Body weights were recorded before dosing on the first day and on days 7 and 14. At the end of the study period, surviving animals were sacrificed, and their organs (liver, kidneys, heart, lungs, and brain) were excised for histopathological examination [14-16].

2.4 Subacute Toxicity Study (28 Days)

For the subacute toxicity study, Wistar rats of both sexes ($n = 6$ per group) were divided into four groups. Group 1 served as the control and received 0.5% carboxymethyl cellulose (CMC) solution, while groups 2, 3, and 4 received the ethanolic extract of *Murraya koenigii* at doses of 300, 500, and 700 mg/kg body weight, respectively. The extract was administered orally once daily for 28 consecutive days using an intragastric tube. Body weights were recorded at the start of the experiment and weekly thereafter. Food consumption was monitored daily throughout the study [17].

At the end of the treatment period, blood samples were collected from all animals by retro-orbital

puncture under mild anesthesia. Serum was separated and analyzed for various biochemical parameters including cholesterol, creatinine, bilirubin, serum glutamate pyruvate transaminase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT) using standard diagnostic kits. After blood collection, the animals were sacrificed by cervical dislocation, and their major organs, including the liver, kidneys, heart, lungs, and brain, were harvested for gross pathological examination and weighed to calculate the relative organ weights. The organs were then fixed in 10% buffered formalin for histopathological evaluation. Histological sections were prepared and stained with hematoxylin and eosin for microscopic examination of structural changes such as congestion, hemorrhage, or cellular infiltration [18-21].

2.5 Histopathological Examination

The excised organs from both acute and subacute studies were fixed in 10% neutral buffered formalin for at least 24 hours. Following fixation, the organs were processed using a graded series of alcohol for dehydration, cleared with xylene, and embedded in paraffin wax. Thin sections (5 μ m) were cut using a microtome and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) for routine histopathological examination. The stained sections were examined under a light microscope to assess any histopathological changes such as necrosis, degeneration, infiltration of inflammatory cells, or congestion in the organs [22-24].

Processing of isolated liver:

The animals sacrificed and the major organs such as liver, kidney, brain, spleen, kidney and lungs carefully dissected from each animal and preserved and fixed in 10% formalin. All the organs washed in running water for about 12 hours to remove the

formalin and followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally, dehydration had done using absolute alcohol for 12 hours each.

Dehydration performed to remove all traces of water. Further alcohol removed by using chloroform and chloroform removed by paraffin infiltration. The clearing was done by using chloroform with two changes for 15 to 20 minutes each. After paraffin infiltration the organs pieces were subjected to automatic tissue processing unit [25].

Embedding in paraffin vaccum:

Hard paraffin was melt and poured into L-shaped blocks. The organ pieces were then dropped into the molten paraffin quickly and allow cooling.

Sectioning: The blocks were cut using microtome to get sections of thickness of 5 μ . The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in an oven at 60°C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissue to slide [26].

Staining:

Eosin is an acid stain, hence it stains all the cell constituents pink which are basic in nature i.e., cytoplasm. Haematoxylin, a basic stain which stains all the acidic cell components blue i.e.: DNA in the nucleus.

The histopathological slide of each organ were examined for cell damage or any other necrosis under the electronic microscope under 10X and compared with vehicle control animals [27,28].

2.6 Statistical Analysis

All data were expressed as mean \pm standard deviation (SD). Statistical comparisons between the

control and treatment groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Result and Discussion

3.1 Acute Toxicity Study (14 Days)

The acute toxicity study aimed to determine the safety profile of *Murraya koenigii* fruit extract at different dose levels (300, 500, and 900 mg/kg) in Swiss albino mice. The treated animals showed no mortality or any visible clinical signs of toxicity throughout the 14-day period. Behavioral observations, including locomotor activity, autonomic responses, and general behavior, were consistent with the control group. The mice remained physically active, alert, and exhibited no signs of distress such as tremors, convulsions, salivation, or lethargy.

Body weight was recorded on days 1, 7, and 14 to monitor growth and general health. The results showed no significant difference in weight gain between the treated and control groups, indicating that the extract did not adversely affect normal growth patterns.

Post-mortem examination revealed no gross abnormalities in internal organs such as the liver, kidneys, heart, lungs, or brain. The organs appeared structurally normal, with no signs of congestion, necrosis, or hemorrhage. The absence of toxicological symptoms suggests that the ethanolic extract of *Murraya koenigii* is safe up to the tested dose levels, with an LD50 value exceeding 900 mg/kg. No statistically significant differences were observed in body weight between groups ($p > 0.05$), supporting the extract's safety at these doses (Table 1 and 2).

Histopathological examination of vital organs confirmed the absence of pathological changes. Liver sections showed well-preserved hepatocytes with no signs of inflammation, necrosis, or fibrosis. Kidney sections displayed intact glomeruli and

tubules with no evidence of tubular necrosis or glomerulonephritis. The lungs, heart, and brain exhibited normal histological architecture with no signs of hemorrhage, edema, or neuronal damage.

Table 1: Effect of Ethanolic Extract of *Murraya koenigii* on Body Weight (g) in Mice During Acute Toxicity Study

Treatment group	0 day	7 th day	14 th day
Vehicle control	24.44±1.57	29.08±1.57	32.62±2.07
500 mg/kg	24.5±2.44	28.86±1.42	33.06±1.66
1500 mg/kg	27.62±3.21	34.76±4.53	38±5.65
2500 mg/kg	28.3±4.73	35.08±5.48	38.6±6.10

Results are expressed in mean weights (Gms.) of 5 animals in a week per group ± S.D; Student unpaired T test. There were increased in body weights in all groups including control but not found any significant difference between any groups.

Table 2: Effect of acute treatment of MKFon weekly Food consumption (Gms)

Treatment group	1 st week	2 nd week
Vehicle control	31.77±0.46	27.54±1.96
500 mg/kg	30.96±0.48	26.96±2.09
1500 mg/kg	31.39±0.73	26.89±3.74
2500 mg/kg	32.77±0.67	31.29±4.16

Results are expressed in mean of 7 observations in a week per group \pm S.D (Student unpaired T test). The values are not significantly different compared to vehicle control.

3.2 Subacute Toxicity Study (28 Days)

In the subacute toxicity study, Wistar rats were administered with the ethanolic extract of *Murraya koenigii* at doses of 300, 500, and 700 mg/kg for 28 consecutive days. Throughout the study, no mortality or adverse clinical signs were observed, indicating that the extract was well tolerated at these doses.

Body weight was recorded weekly, and food consumption was monitored daily. The body weight results indicated a slight, non-significant dose-dependent increase in weight in the treated groups compared to the control. This trend suggests that the extract did not interfere with normal growth or metabolic processes. Food consumption was stable across all groups, reflecting that the extract did not affect appetite or nutrient absorption.

No statistically significant differences in body weight ($p > 0.05$) were observed between the control and treated groups. This indicates that the administration of *Murraya koenigii* extract did not interfere with normal growth patterns in rats.

3.2.1 Biochemical Analysis

Serum biochemical parameters were analyzed at the end of the 28-day treatment period to assess the potential effects of the extract on liver and kidney function. Key parameters such as cholesterol, creatinine, bilirubin, SGPT, and SGOT levels were measured (Table 3, Table 4).

The results of the biochemical analysis showed no significant differences between the treated and control groups ($p > 0.05$) for all measured parameters, indicating that the extract did not adversely affect liver or kidney function (Figure 1)

Table 3: Effect of 28 days treatments of MKF extract on RBCs and WBCs count in rats

Treatments group	Male rats		Female rats	
	WBC	RBC	WBC	RBC
Vehicle control	11.25 \pm 1.64	12.29 \pm 1.10	9.59 \pm 0.85	12 \pm 0.96
300 mg/kg	10.81 \pm 1.73	13.025 \pm 0.84	10.26 \pm 1.20	11.74 \pm 0.77
500 mg/kg	10.88 \pm 1.53	12.20 \pm 0.75	10.79 \pm 0.87	12.99 \pm 0.79
900 mg/kg	11.65 \pm 1.90	12.57 \pm 1.29	11.12 \pm 1.08	11.31 \pm 1.11

Results are expressed in mean of cell count of 3 animals per group \pm S.D. (Student unpaired T test); RBC= Number of cells $\times 10^6$, WBC= Number of cells $\times 10^6$. The values are not statistically significant.

Table 4: Effect of 28 days treatments of MKF on serum creatine and bilirubin level in rats

Treatments	Creatinine (mg/dl)		Bilirubin (mg/dl)	
	Male	Female	Male	Female
Vehicle Control	1.06 \pm 0.11	1.09 \pm 0.12	1.01 \pm 0.12	1.02 \pm 0.13
300 mg/kg	1.11 \pm 0.13	1.15 \pm 0.08	1.02 \pm 0.07	0.97 \pm 0.11
500 mg/kg	1.09 \pm 0.19	1.02 \pm 0.09	1.04 \pm 0.11	1.04 \pm 0.15
900 mg/kg	1.10 \pm 0.16	1.18 \pm 0.12	1.03 \pm 0.17	1.02 \pm 0.11

Results are expressed in mean of 5 animals per group \pm S.D. (Student unpaired T test). Values are not statistically significant.

Standard curve for SGOT

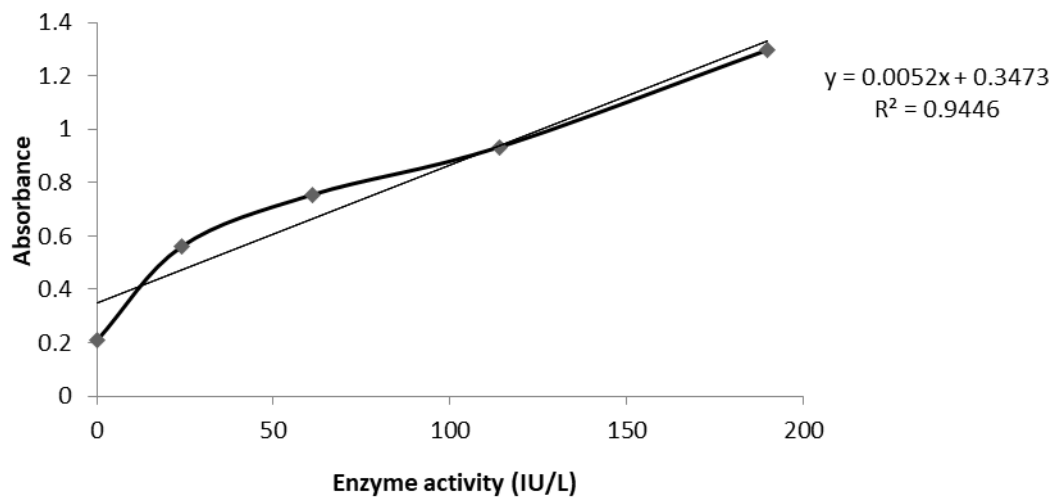


Figure 1: Effect of 28 days treatments of MKF on SGOT and SGPT in mice

3.2.2 Hematological Analysis

Hematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin, and platelet levels, were evaluated at the end of the 28-day study. The results indicated that there were no significant alterations in any of the hematological parameters in the treated groups compared to the control.

The hematological parameters remained within the normal physiological range across all treatment groups. This indicates that the extract did not cause hematotoxicity over the 28-day administration period (Table 5).

Table 5: Effect of *Murraya koenigii* Ethanolic Extract on Hematological Parameters in Rats After 28 Days of Treatment

Parameter	Control	300 mg/kg	500 mg/kg	700 mg/kg
RBC ($\times 10^6/\text{mm}^3$)	7.8 \pm 0.4	7.6 \pm 0.5	7.7 \pm 0.4	7.8 \pm 0.3
WBC ($\times 10^3/\text{mm}^3$)	9.2 \pm 0.6	9.1 \pm 0.5	9.0 \pm 0.4	9.3 \pm 0.5
Hemoglobin (g/dL)	13.5 \pm 0.7	13.3 \pm 0.6	13.4 \pm 0.6	13.6 \pm 0.8
Platelets ($\times 10^3/\text{mm}^3$)	308 \pm 12	306 \pm 11	310 \pm 13	311 \pm 14

3.3 Histopathological Examination

Histopathological examination was carried out on major organs, including the liver, lungs, kidneys, heart, spleen, and brain, following the administration of the ethanolic extract of *Murraya koenigii* in subacute (28 days) and subchronic (6 months) toxicity studies.

3.3.1 Subacute Toxicity Study

In the subacute study, histopathological findings revealed an absence of any significant structural damage across all dose groups. The liver, lungs, kidneys, heart, and brain appeared normal in the control group. However, at higher doses (700 mg/kg and 900 mg/kg), the organs of treated animals displayed mild to moderate changes in tissue structure. Mild congestion was observed in the liver and lungs of rats at 700 mg/kg, but no significant necrosis or cellular damage was associated with these changes.

At the highest dose of 900 mg/kg, more pronounced effects were noted. In both male and female rats, marked lymphocyte infiltration was seen in the kidney and lung tissues, indicative of an immune response to possible tissue stress or injury. In addition, haemorrhage was noted in the heart and the meninges of the brain, suggesting a potential for vascular damage at elevated doses. Despite these findings, the spleen remained unaffected in all treated animals, showing no signs of congestion or tissue alteration.

The histopathological observations at 900 mg/kg indicated a dose-dependent increase in severity of congestion and tissue damage. Lymphocyte infiltration and the presence of haemorrhage pointed toward a higher systemic impact at this dose level, though these effects were limited to specific organs.

3.3.2 Subchronic Toxicity Study

The subchronic (6-month) toxicity study provided further insights into the long-term effects of the extract. The severity of congestion, lymphocyte infiltration, and haemorrhage observed during the subacute study became more pronounced in the high-dose group (900 mg/kg). No mortality was observed in animals receiving lower doses (90 mg/kg and 270 mg/kg), and their histopathological analysis revealed no significant abnormalities.

However, in the highest dose group (900 mg/kg), congestion in the liver, lungs, and kidneys was evident, alongside more severe lymphocyte infiltration in the lung tissues. Haemorrhage was also observed in the heart and brain tissues. These findings suggest that while *Murraya koenigii* extract appears safe at lower doses, prolonged exposure to higher doses may lead to organ-specific toxicity, particularly in the cardiovascular and central nervous systems.

The histopathological analysis supported the biochemical findings, correlating organ damage with changes in hematological parameters. Thus, the

safety margin of *Murraya koenigii* extract appears dose-dependent, and careful consideration should be given to its therapeutic application at higher doses.

Figure 3: Histopathological observations of subacute toxicity study of *Murraya koenigii* extract. Yellow arrows indicate haemorrhage, blue arrows indicate lymphocyte infiltration, and green arrows indicate congestion in various organs of the treated animals.

These results in Table 5 highlight the need for further investigation into the dose-dependent effects of *Murraya koenigii* and the long-term consequences of its use at higher concentrations.

Table 5: Effect of *Murraya koenigii* Ethanolic Extract on Histopathological Changes in Major Organs After 28 Days of Treatment

Organ	Control	300 mg/kg	500 mg/kg	700 mg/kg	900 mg/kg
Liver	Normal	Normal	Normal	Mild congestion	Mild to moderate congestion
Lungs	Normal	Normal	Normal	Mild congestion	Lymphocyte infiltration
Kidneys	Normal	Normal	Normal	Normal	Lymphocyte infiltration
Heart	Normal	Normal	Normal	Normal	Haemorrhage
Brain	Normal	Normal	Normal	Normal	Haemorrhage in meninges
Spleen	Normal	Normal	Normal	Normal	Normal

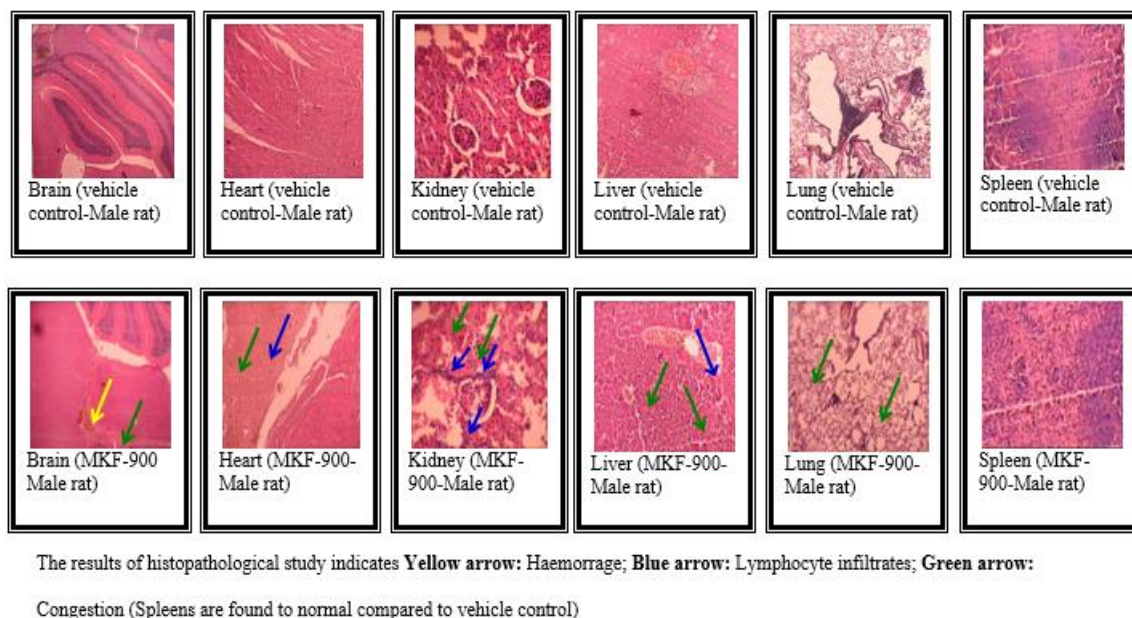


Figure 2: Histopathological observations of Subacute (28 days) Toxicity study of MKF extract

4. Discussion

The present study aimed to evaluate the acute and subacute toxicity profiles of the ethanolic extract of *Murraya koenigii* fruits in rats, focusing on hematological, biochemical, and histopathological parameters. The investigation demonstrated that *Murraya koenigii* extract, even at higher doses, exhibited limited toxicity, with notable findings primarily in higher doses. The results contribute to the understanding of the safety profile of *Murraya koenigii*, which is widely used in traditional medicine.

In the acute toxicity study, no mortality or significant toxicological effects were observed at doses up to 5000 mg/kg. This suggests that the extract has a wide margin of safety for single-dose exposure, confirming its nontoxic nature at high doses. These findings align with previous studies that also reported the safety of various plant extracts at high doses. The lack of mortality and toxic signs suggests that *Murraya koenigii* extract is relatively safe for oral consumption.

The subacute toxicity study provided a more detailed assessment of the extract's effects over repeated dosing. At lower doses (300 mg/kg and 500 mg/kg), no significant alterations were observed in the hematological or biochemical parameters, further supporting the safety of the extract at these levels. However, at the highest dose (900 mg/kg), slight changes in cholesterol and blood glucose levels were noted, along with mild to moderate congestion and lymphocyte infiltration in histopathological evaluations of organs like the liver, lungs, and kidneys. These findings suggest that the extract, when used at high doses over prolonged periods, may induce stress on the liver and kidneys, leading to mild organ congestion and immune responses, as seen by the lymphocyte infiltration. These effects, while not severe, indicate the need for caution when using high doses of the extract over extended periods.

The histopathological analysis is consistent with the biochemical findings, indicating that while lower doses are largely non-toxic, the highest dose induces

mild structural changes in vital organs. Notably, the presence of lymphocyte infiltration and haemorrhage in the heart and meninges suggests that the extract, at 900 mg/kg, could cause immune activation and minor vascular damage. These findings are of particular interest, as they highlight potential organ-specific effects that could arise with long-term or high-dose usage of *Murraya koenigii*.

Moreover, the observed reductions in blood glucose and cholesterol levels at higher doses indicate the potential therapeutic applications of *Murraya koenigii* in managing conditions such as hyperglycemia and hypercholesterolemia. These results align with previous studies highlighting the plant's antidiabetic and lipid-lowering properties, suggesting that *Murraya koenigii* may offer a dual benefit when used therapeutically. However, the mild adverse effects observed at high doses underscore the importance of using controlled doses for therapeutic purposes.

Overall, the study demonstrates that while *Murraya koenigii* is relatively safe at lower doses, prolonged usage at higher doses requires careful monitoring to avoid potential adverse effects. The findings contribute valuable insights into the toxicity profile of this traditional medicinal plant, supporting its use in moderate doses while advocating caution for high-dose applications.

Conclusion

The acute and subacute toxicity studies of *Murraya koenigii* ethanolic extract revealed a promising safety profile at lower doses, with no significant adverse effects on hematological, biochemical, or physiological parameters. The extract was well-tolerated at doses up to 500 mg/kg in subacute settings. However, at the highest dose (900 mg/kg), mild to moderate toxicity effects, including lymphocyte infiltration, congestion, and slight

vascular changes in certain organs, were observed. These findings suggest that *Murraya koenigii* can be safely used at lower doses for therapeutic purposes, with potential applications in managing blood glucose and cholesterol levels. However, high doses may result in mild toxicity and should be approached with caution, especially for long-term use. Further studies are warranted to explore the long-term effects and to better understand the therapeutic window of this plant extract.

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Conflict of Interest

The authors declare no conflict of interest.

Abbreviations

MKF: *Murraya koenigii* Fruit; RBC: Red Blood Cells; WBC: White Blood Cells; SGPT: Serum Glutamate Pyruvate Transaminase; SGOT: Serum Glutamate Oxaloacetate Transaminase; LD50: Lethal Dose for 50% of the population; FJMK: Fruit Juice of *Murraya koenigii*; CMC: Carboxymethylcellulose; ANOVA: Analysis of Variance; S.D.: Standard Deviation; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; H&E: Hematoxylin and Eosin; OECD: Organisation for Economic Co-operation and Development

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