

INVESTIGATING THE IMPACT OF 28-DAYS ORAL EXPOSURE OF ETHANOLIC EXTRACT OF MURRAYA KOENIGII FRUITS IN WISTAR RATS

Atish N Waghmare¹, Jagdish V Manwar², Yashpratapsingh Rana³, Bharat V Dhokchawle⁴, Pravin G Morankar⁵, Shriram H. Bairagi⁶

^{1,2}Department of Pharmaceutical Chemistry, Kamalprakash Pharmacy College and Research Centre, Karanja, India

³School of Pharmacy, Glocal University, Saharanpur (U.P) India

^{4,5}St John Institute of Pharmacy and Research, Vevoor, Manor Road, Palghar (E), Dist-Palghar-401404, Maharashtra, India.

⁶YNP College of Pharmacy, Asangaon, Palghar, Maharashtra, 401103

Abstract

Murraya koenigii fruits extract was prepared and investigated for repeated dose toxicity in male and female rats. Three different doses at 250, 500, and 750 mg/kg of ethanolic extract of Murraya koenigii fruit were administered daily to groups of 10 animals (five males and five females) for 28 days. A control group received the vehicle (0.5% CMC). Various biochemical parameters, including RBC, WBC, glucose, haemoglobin, cholesterol, creatinine, bilirubin, SGPT, and SGOT, were measured across all groups. In addition, physiological parameters such as daily food intake, weekly body weight, visual and auditory function, and organ weights were recorded. The results indicated that none of the animals in any dose group exhibited test material-related changes in RBC, WBC, creatinine, bilirubin, SGPT, or SGOT. Similarly, no significant changes were observed in physiological parameters like food consumption, visual, or auditory function. However, some changes were noted, including an increase in haemoglobin levels, significant weight loss, and a decrease in cholesterol and glucose levels in the animals. Rats in the 500 mg/kg and 750 mg/kg groups exhibited a loss of subcutaneous fat during the final two weeks of treatment. These groups also showed reductions in total cholesterol and glucose levels, with the most significant effects seen at the 750 mg/kg dose. Based on these findings, the study concludes that the administration of ethanolic Murraya koenigii leaf extract for 28 days increases haemoglobin levels, reduces body weight, subcutaneous fat, and blood glucose levels in medium and high-dose groups.

Keywords: Murraya Koenigii Fruit, Repeated dose toxicity study, Histopathology

Introduction

Murraya koenigii (L.), a member of the Rutaceae family, is an aromatic, semi-deciduous shrub or small tree that can grow up to 6 meters in height and is found throughout India. Commonly known as "Meethi neem" or "Karry tree," it has been traditionally used for its antiemetic, antidiarrheal, febrifuge, and blood-purifying properties. The entire plant is regarded as a tonic and stomachic. Its leaves are frequently used as a flavouring in curries and chutneys, and nearly every part of the plant exudes a strong, distinctive odour. In particular, people in the plains, especially in Southern India, incorporate the leaves as a spice in various curry dishes [1,2]. In this study, the fruits of Murraya koenigii were selected, as they are

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renowned for their potential in treating anaemia, as well as exhibiting antioxidant, antiulcer, and anthelmintic activities, as demonstrated in our previous research [3-6]. *Murraya koenigii* is widely used not only as a flavouring and condiment but also in folk medicine for treating various metabolic and infectious diseases. The leaves, bark, roots, and fruits have long been used in traditional medicine, especially for alleviating stomach aches, serving as a stimulant, and as a tonic for vitality [7]. Phytochemical analysis of *M. koenigii* has revealed the presence of vitamins, carbazole alkaloids, terpenoids, phenolic compounds, and essential minerals such as calcium, iron, zinc, and vanadium. Additionally, the carbazole alkaloids found in *M. koenigii* are reported to possess antioxidant and antidiabetic properties [8-11]. Numerous studies have highlighted the biological activities of *M. koenigii* leaves, including its anti-hypercholesterolaemic effects [12,13] and its potential in preventing colon carcinogenesis [14]. It has also been documented for its antimicrobial and antioxidant activities [15-18]. Despite these notable pharmacological properties, there is no available report on the toxicological evaluation of *Murraya koenigii* fruit extracts in the literature.

Plants have played a vital role in traditional medicine due to their therapeutic and, in some cases, toxic properties [19-20]. The use of any medicinal drug is often based on long-term clinical experience. Medicinal plants hold significant importance in public health, particularly among low-income populations with limited access to modern medicine [21-25]. However, there is insufficient scientific evidence to fully support the safety and efficacy of many herbal remedies. Given the medicinal value of *Murraya koenigii*, this study aimed to evaluate the toxicity profile of its fruit extract on haematological and physiological parameters. To the best of our knowledge, there is no prior literature on the toxicity profile of *Murraya koenigii* fruits. Repeated-dose toxicity data are essential for assessing the safety and long-term effects of medicinal plants. Thus, this study was designed to investigate the toxicological effects of *Murraya koenigii* following 28 days of repeated-dose administration. and the toxicological effects of long-term administration of *Murraya Koenigii* through a 28-day repeated dose toxicity evaluation.

Materials and Methods

Murraya koenigii fruits were gathered from the Yavatmal district in Maharashtra, during the period of June to September 2021. Dr. N. M. Dongarwar, Head of the Botany Department at RTM Nagpur University in Nagpur, authenticated the fruits. The Herbarium, Department of Botany, RTM Nagpur University, Nagpur, India, received a voucher specimen (No. 9916). Biochemical estimation kits were utilized, including RBC and WBC diluting fluids, Drabkin's reagent for haemoglobin estimation (AGAPPE Diagnostics), and kits for serum creatinine, total bilirubin, SGOT, and SGPT estimation (Biolab Diagnostic Pvt. Ltd.).

Experimental Animals

The study was conducted on male and female Wistar rats weighing between 100–110 g. The animals were maintained on a standard diet (Amrut Feed, Sangli, Maharashtra) with free access to water under a well-ventilated 12-hour light-dark cycle. They were acclimatized to laboratory conditions for seven days before the experiments. All procedures involving animals followed the guidelines of the Organization for Economic Cooperation and Development (OECD) No. 407 (1993) and were approved by the Institutional Animal Ethics Committee (IAEC) of S.N. Institute of Pharmacy, Pusa, Maharashtra.

Preparation of extracts of *Murraya koenigii* fruits

The gathered *Murraya koenigii* fruits were dried in the shade, grounded into a powder in an electric blender, and then extracted using Soxhlet's extractor with distilled ethanol as a solvent in a 1:4 ratio (50g powder and 200ml solvent). The extraction was carried out for around eighteen hours, or until the solvent in the thimble turned clear, signifying that it was finished. The extract was employed in the current investigation for pharmacological and phytochemical evaluation after it was fully extracted and concentrated by evaporation at room temperature. Extraction was done in several batches and product from all the batches was found to be similar in appearance.

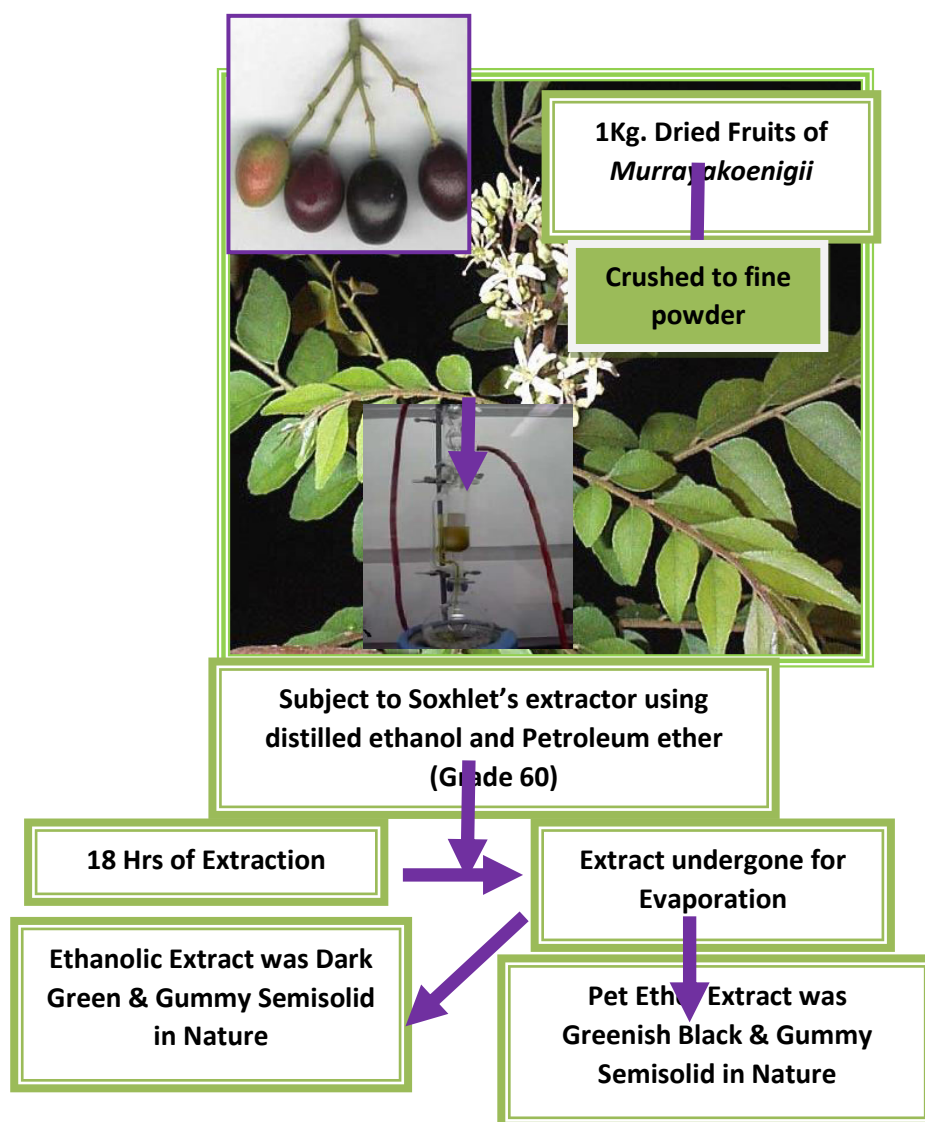


Figure 1: Flow chart for the Extraction process for Fruits of *Murraya koenigii*

Qualitative Chemical Tests/Phytochemical Investigations of extracts of *Murraya koenigii* fruits: According to the findings of a qualitative chemical analysis of *Murraya koenigii* fruits, the following components are present in ethanolic extracts. The findings are displayed in table 1

Table 1: Phytochemical Investigations of extracts of *Murraya koenigii*

Sr No.	Test for phytoconstituents	Result
01	Tests for Carbohydrates	Positive
02	Test for Gums	Positive
03	Test for Mucilage	Positive
04	Tests for proteins	Positive
05	Test for Fats and Oils	Positive
06	Test for Sterols and Triterpenoids	Positive
07	Tests for Glycosides	Positive
08	Tests for Alkaloids	Positive
9	Tests for Saponins	Negative
10	Tests for Flavonoids	Positive

Toxicity Studies

Wistar rats of both sexes (80–100 g) were assigned to groups, with each group consisting of five animals of each sex. A total of four groups were formed per sex. Group I received 0.5% CMC for 28 days, while Groups II, III, and IV were administered 250 mg/kg, 500 mg/kg, and 750 mg/kg of MKF extract orally, respectively. Body weight was recorded at the beginning of the study and subsequently at weekly intervals.

Evaluation of Biochemical Parameters

At the end of the 28-day study, the animals were dissected, and blood samples were collected first from the retro-orbital plexus and then from the posterior vena cava under light ether anaesthesia. Haematological analysis was conducted to determine the total RBC and WBC counts. Additionally, haemoglobin levels, SGOT, SGPT, total bilirubin, serum creatinine, and total cholesterol in the serum were measured using commercial kits.

Evaluation of Physiological parameters

Daily food consumption, weekly body weight, locomotor activity, grip strength, and auditory and visual responses were assessed at the conclusion of the study. Following the experiment, the animals were sacrificed, and the liver, lungs, kidneys, spleen, brain, and heart were carefully dissected to determine their absolute weights. The relative organ weight for each animal was then calculated. After weighing, the organs were preserved in a 10% formalin solution and examined for structural abnormalities through histopathological analysis.

Results and Discussion

Acute toxicity testing of the ethanolic extract of *Murraya koenigii* revealed it to be safe up to a dose of 850 mg/kg, beyond which mortality was observed in animals. Consequently, the highest dose selected for the repeated-dose study was 750 mg/kg. The LD50 was determined to be 2500 mg/kg, with 1/10th of this dose (250 mg/kg) considered an effective dose. Therefore, 250 mg/kg was included in the study to assess its toxic effects. After 28 days of repeated administration, no significant changes were observed in red blood cell (RBC) and white blood cell (WBC) counts at any dose level. Similarly, total serum bilirubin, serum creatinine, SGOT, and SGPT levels remained unchanged. However, haemoglobin levels increased at medium and high doses, with a statistically significant ($p < 0.05$) increase at 700 mg/kg in both male and female rats (Table 1).

Table 2: Influence of MKF treatments on biochemical parameters. Results are expressed in mean of 5 animals per group $\pm$ S.D; *Significantly different compared to control at $p < 0.05$ by using student unpaired T test.

Parameters	Sex	Control	MKF -250	MKF -500	MKF -750
Bilirubin (mg/dl)	Male rats	1.05 $\pm$ 0.17	1.09 $\pm$ 0.096	1.08 $\pm$ 0.09	1.08 $\pm$ 0.19
	Female rats	1.12 $\pm$ 0.18	0.87 $\pm$ 0.14	1.10 $\pm$ 0.20	0.87 $\pm$ 0.18
Hb (gm/dl)	Male rats	13.56 $\pm$ 1.39	15.48 $\pm$ 0.98	17.84 $\pm$ 0.984	15.86 $\pm$ 0.72*
	Female rats	12.69 $\pm$ 0.84	12.37 $\pm$ 0.87	13.8 $\pm$ 0.82	13.50 $\pm$ 0.98*
Creatinine	Male rats	1.14 $\pm$ 0.12	1.16 $\pm$ 0.10	1.29 $\pm$ 0.21	1.19 $\pm$ 0.164
	Female rats	1.14 $\pm$ 0.22	1.25 $\pm$ 0.8	1.26 $\pm$ 0.19	1.18 $\pm$ 0.20
RBC	Male rats	13.64 $\pm$ 1.08	14.25 $\pm$ 0.87	13.25 $\pm$ 0.79	13.64 $\pm$ 1.89
	Female rats	13.08 $\pm$ 0.84	15.47 $\pm$ 0.82	11.56 $\pm$ 0.89	14.42 $\pm$ 1.24
WBC	Male rats	12.45 $\pm$ 2.04	11.84 $\pm$ 1.90	12.48 $\pm$ 2.04	13.75 $\pm$ 2.30
	Female rats	10.08 $\pm$ 1.09	11.29 $\pm$ 1.34	11.84 $\pm$ 0.84	13.22 $\pm$ 1.14
SGPT	Male rats	0.61 $\pm$ 0.09	0.81 $\pm$ 0.09	0.52 $\pm$ 0.09	0.83 $\pm$ 0.09
	Female rats	0.72 $\pm$ 0.14	0.65 $\pm$ 0.92	0.71 $\pm$ 0.14	0.74 $\pm$ 0.12
SGOT	Male rats	0.69 $\pm$ 0.75	0.99 $\pm$ 0.94	0.87 $\pm$ 0.89	0.84 $\pm$ 0.92
	Female rats	0.58 $\pm$ 0.18	0.84 $\pm$ 0.14	0.59 $\pm$ 0.71	0.6865 $\pm$ 0.21

Additionally, at 750 mg/kg, a significant ($p < 0.05$) reduction in blood glucose and cholesterol levels was observed in both male and female rats, while at 500 mg/kg, only male rats exhibited a significant decrease in cholesterol levels (Figures 2 and 3). These findings suggest potential hypoglycemic and hypocholesterolemic effects of *Murraya koenigii*. The observed

reductions in glucose and cholesterol levels align with previous studies on *M. koenigii* leaves, which demonstrated anti-diabetic and cholesterol-lowering properties.

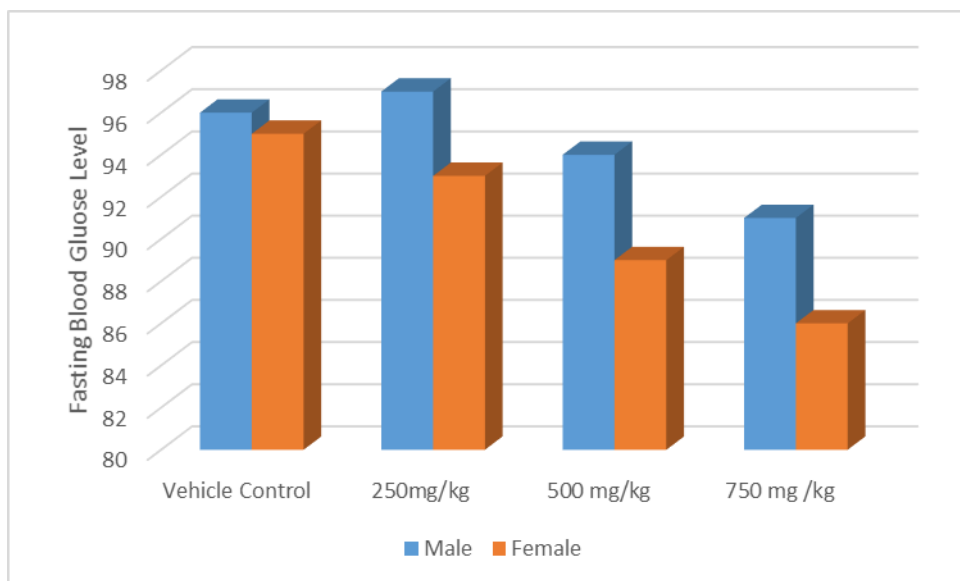


Figure 2: Effect of 28 days' treatments of MKF on blood glucose level in male and female rats Results are expressed in mean of 5 animals per group $\pm$ S.D; *

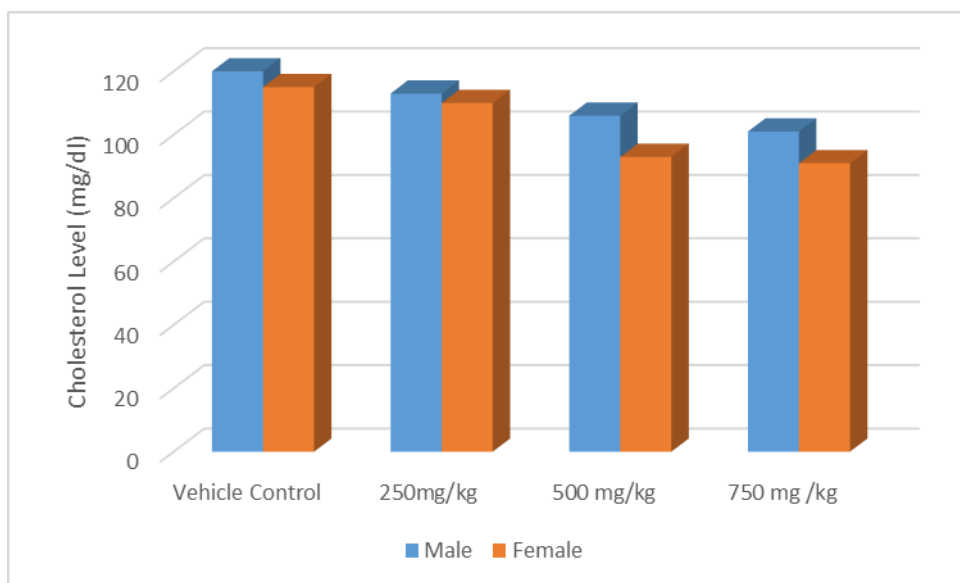


Figure 3: Effect of 28 days' treatments of MKF on Cholesterol level in male and female rats Results are expressed in mean of 5 animals per group $\pm$ S.D; *

The study also indicated a marked weight loss in male rats at doses of 500 and 750 mg/kg after the second week of administration, likely due to the loss of subcutaneous fat and a decrease in total cholesterol levels. A significant ($p < 0.05$) decrease in body weight and fat loss was observed in the 750 mg/kg group in the final week compared to the second week. Female rats exhibited a slight decrease in body weight, though it was not statistically significant (Figures 4 and 5)

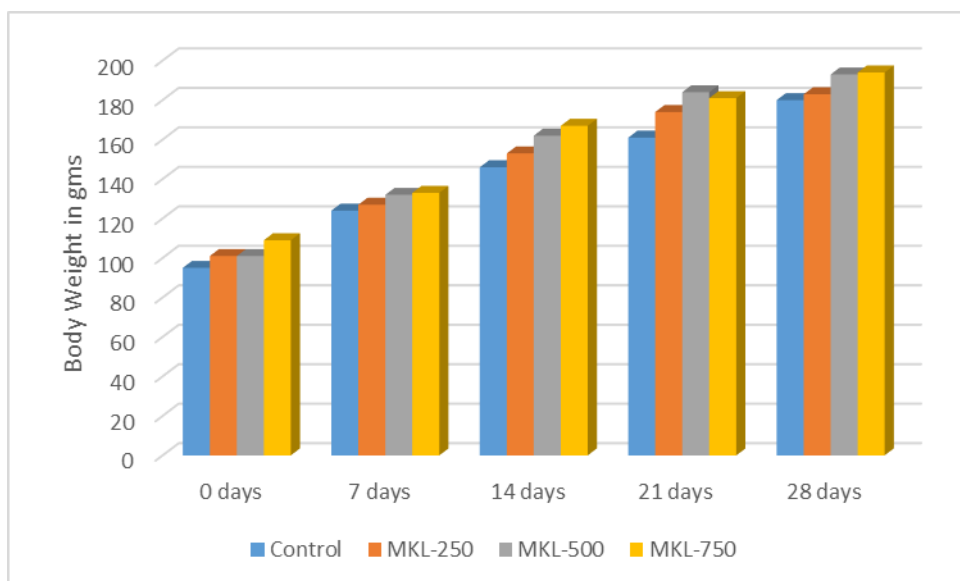


Figure 4: Effect of MKF on Body weight in male rats Results are expressed in mean of 5 animals per group $\pm$ S.D.

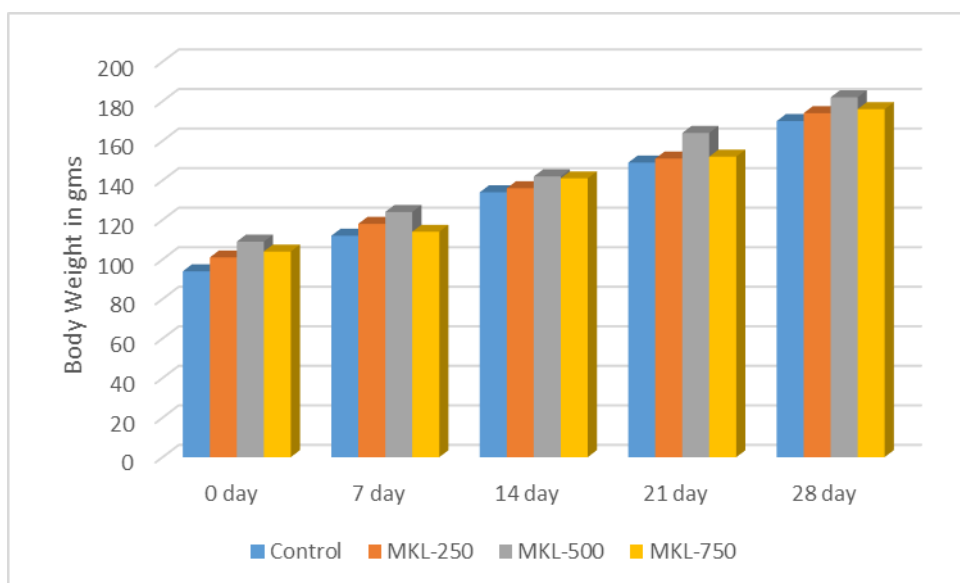


Figure 5: Effect of MKF on Body weight in female rats Results are expressed in mean of 5 animals per group $\pm$ S.D.

Food consumption increased across all groups, including the vehicle control, indicating that *Murraya koenigii* fruit extract had no significant impact on appetite. Furthermore, no significant ($p < 0.05$) effects were observed on other physiological parameters, such as auditory and visual responses (Table 3).

Table 3: Results are expressed in mean of 5 animals per group $\pm$ S.D; * indicates significant ($p < 0.05$) different compared to vehicle control readings.

Rat	Physiological response	Control	250 mg/kg	500 mg/kg	750 mg/kg
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Male	Auditory	Pass	Pass	Pass	Pass
	Visual	Pass	Pass	Pass	Pass
	Locomotor activity (sec.)	85.230± 11.48	112.32 ± 8.9	79.41 ±12.21	101.59 ± 14.24
	Grip strength (sec.)	164.21±11.32	161.08±7.89	153.97± 10.22	167.37± 5.34*
Female	Auditory	Pass	Pass	Pass	Pass
	Visual	Pass	Pass	Pass	Pass
	Locomotor activity(sec.)	121.4 ± 19.21	114.21 ± 13.22	105.42± 14.86	109.27 ±11.65
	Grip strength (sec.)	152.4± 34.84	134.4 ± 31.85	141.4 ±20.14	135.6 ±29.99*

After 28 days of repeated exposure, no signs of mortality or morbidity were observed at any dose level, except for congestion, hemorrhage, and lymphocyte infiltration at the highest dose (750 mg/kg) in both male and female rats (Figure 4). No such pathological changes were noted at doses of 250 mg/kg and 500 mg/kg, indicating their safety. The safety profile of *Murraya koenigii* fruit extract was further supported by organ weight ratios, which remained stable across all groups (Table 4).

Table 4: Organ weight ratios after administration of MKF extract. Results expressed as mean of relative organ weight of 5 animals per group ± S.D.

Organs	Sex	Control	MKF-250	MKF-500	MKF-750
Liver	Male rats	2.21±0.31	2.41±0.50	2.73± 0.29	3.54± 0.39
	Female rats	2.7±0.32	2.47±0.28	2.69±0.42	3.20±0.41
Lung	Male rats	1.18± 0.22	0.94±0.10	1.13±0.19	1.97±0.19
	Female rats	0.99±0.96	1.21±0.18	1.34±0.19	1.15±0.24
Heart	Male rats	0.36±0.11	0.31±0.09	0.51±0.31	0.39±0.10
	Female rats	0.28±0.19	0.31±0.19	0.31±0.14	0.29±0.31
Spleen	Male rats	0.19±0.28	0.21±0.19	0.19±0.11	0.17±0.15
	Female rats	0.15±0.20	0.19±0.14	0.18±0.09	0.17±0.10
Brain	Male rats	0.89±0.14	0.84±0.16	1.24±0.30	1.18±0.24
	Female rats	0.98±0.10	0.94±0.17	1.03±0.30	1.10±0.12
Kidney	Male rats	0.61± 0.13	0.61±0.12	0.61±0.20	0.72±0.21
	Female rats	0.46±0.12	0.64±0.15	0.51±0.18	0.56±0.61

Conclusion:

Based on these findings, the study concludes that the long-term administration (28-day repeated-dose toxicity study) of ethanolic *Murraya koenigii* fruit extract does not cause toxic effects on haematological or physiological parameters. However, it increases haemoglobin levels and decreases body weight, subcutaneous fat, blood glucose, and cholesterol levels at medium and high doses. The study also indicated a marked weight loss in male rats at doses

of 500 and 750 mg/kg after the second week of administration, likely due to the loss of subcutaneous fat and a decrease in total cholesterol. These effects highlight its potential therapeutic applications in treating anaemia, managing diabetes, and lowering cholesterol. Furthermore, the study confirms that *Murraya koenigii* is safe for consumption up to a dose of 500 mg/kg without causing structural damage to organs.

Conflict of Interest: The authors declare that they have no conflict of interest.

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