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Pharmacological safe dose assessment of *Mangifera indica* Linn. leaves extract according to the Organization for Economic Cooperation and Development (OECD) 420 standards

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Mangifera indica Linn. leaves extract owns various medicinal properties including antioxidant, anti-diabetic, anti-cancerous, and anti-inflammatory activities. The aim of this study is to find out the safe oral dose of ethanolic extract of *M. indica* leaves on Swiss albino mice for pharmacological purpose. The Organization for Economic Cooperation and Development (OECD) Guideline 420 was followed to assess the acute oral toxicity. *M. indica* leaf extract was administered in a dose dependent manner orally at dosages of 50-, 300-, and 2000- mg kg^{-1} body weight (b.w.) in the sighting study, with one animal used for each dosage. Based on the sighting study, the highest dose of 2000 mg kg^{-1} b.w. of *M. indica* leaves extract was selected for the main study. Continuous monitoring for successive 14 days was done for any behavioural sign of toxicity. Body weight and relative organ weight and biochemical parameters were assessed, and gross necropsy was performed on 15th day. Further, hematoxylin and eosin (H&E) staining of the liver, kidney and testes was performed. The body weight was significantly increased in both studies without any major changes in relative organ weight (ROW), and histology of H&E-stained tissues, wherein no obvious signs of toxicity and mortality were seen. The results of this study suggest that the *M. indica* leaves extract can be categorized as unclassified according to the Globally Harmonised Classification System for chemical substances and mixtures. Therefore, our study concludes that ethanolic extract of *M. indica* leaves less than 2000 mg kg^{-1} b.w. can be considered safe for traditional therapeutic uses.

Keywords: Acute toxicity, Alkaloids, Hepatotoxicity, *Mangifera indica*, OECD, Pharmacological

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Ayurveda is one of the most respected systems of traditional medicine, gained global acceptance in recent years for its uniqueness and natural ways to treat disease and promote healthcare[1]. Ayurvedic medicine system adopts a holistic approach towards physical health and mental well-being, environmental considerations, dietary and lifestyle habits, and treating and managing various diseases[2]. However, in use of traditional medicine, the fixed effective doses have not been scientifically figured out with certainty. Moreover, to ensure the safety and efficacy of the plant extracts, toxicity studies are must criteria for their acceptable usage globally.

M. indica (Family: Anacardiaceae) is an evergreen tree, its plant parts such as leaves, flowers, fruits, stem and bark having various traditional medicinal usage, in addition being one of the most economically important tropical fruit crops[3]. Micronutrients, prebiotic dietary fibre, vitamins, minerals and poly-phenolic flavonoid compounds (beta carotene and beta-cryptoxanthin) and other phytochemicals are important constituents of *M. indica*[4].

Recent studies reported the versatile pharmacological effects of *M. indica* Linn., which includes antioxidant, anti-diabetic, anti-tumour, radio-protective, immunomodulatory, anti-allergic, anti-inflammatory, anti-viral, lipolytic, anti-microbial, anti-bacterial, anti-parasitic and anti-diarrhoeal effects[3]. Besides several pharmacological properties, *M. indica* extracts also have essential nutritional components including proteins as a major bio-macromolecule and oil contents which are responsible for its pleasant aroma due to the presence of active terpenes. It also has vitamins (A, B, C, and E) and other important minerals such as iron (Fe), sodium (Na), magnesium (Mg), potassium (K), phosphorus (P), nitrogen (N), calcium (Ca), boron (B), zinc (Zn) and manganese (Mn)[5]. These minerals play important

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roles, such as maintenance of healthy bones and teeth, immune system regulation, blood pressure regulation, nerve functioning, contraction and relaxation of muscles, blood clotting, energy metabolism and as cofactors for various enzymes[6].

Pan *et al.*[7] reported that mangiferin is the most active phytochemical and main contributor to many of the biological activities of *M. indica* extract. Other phytochemicals include polyphenols, sterols, carotenoids, terpenoids, benzophenones and antioxidants such as flavonoids, quercetin, isoquercetin, ascorbic acid and tocopherols. Out of these, total phenolic compounds (TPC) contribute to numerous physiological processes such as enzymatic activity, cellular redox potential to fight against chronic pathologies, cell proliferation and signal transduction pathways[4]. Philip[8], has shown that *M. indica* leaves are used for the biosynthesis of well-dispersed silver nanoparticles[8]. A recent study has shown no sign of genotoxicity of aqueous extract from *M. indica*, when administered orally for a short term[9].

The main aims of the Organization for Economic Co-operation and Development (OECD) guidelines are first, to reduce the use of laboratory animals by avoiding considering death of animals as an endpoint, second, to speed up chemical safety assessment and third, to enhance the protection of human health and the environment. OECD Guidelines are based on the principle that the predicted toxic doses should be avoided in the main study and the doses which are found to be moderately toxic should be considered for further investigation. Therefore, this OECD compliant was separated into two parts: the sighting study and the main study.

Moreover, in the current study, the acute oral toxicity – fixed dose procedure was followed to investigate the toxicity of ethanol extract of *M. indica* leaves and to standardize the non-toxic doses.

Materials and Methods

Plant material

The *M. indica* leaves were collected from the University of Lucknow (old campus), located in Lucknow, India (latitude 26°51′56″N and longitude 80°56′11″E). Plant samples (accession no. 8346) were identified by CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plants), Lucknow, Uttar Pradesh.

Preparation of crude extract

The *M. indica* leaves were washed thoroughly and shade dried. Then, the leaves were crushed and reduced to a coarse powder with a grinder. Leaves powder was percolated in 95% ethanol by employing Soxhlet apparatus[10] and then evaporated using a vacuum rotary evaporator to concentrate at 60^{o}C in a water bath. The extract was kept and stored at -20^{o}C for further usage.

Phytochemical screening

M. indica leaves extract was qualitatively screened to show the presence of alkaloids, flavonoids, phenols, steroids, glycosides, terpenoids, saponins and tannins using standard methods[11,12].

Estimation of alkaloids: Mayer's reagent test

Two mL of the *M. indica* leaves extract was added to 4.0 mL of Mayer's reagent. A dull white colour precipitate indicated the presence of alkaloids in the extract.

Estimation of flavonoids: alkaline reagent test

One mL of the *M. indica* leaves extract and 4 mL of 4% NaOH solution were mixed in a test tube. A bright yellow colour was developed. After adding a few drops of diluted HCl, the yellow colour turned colourless, indicated the presence of flavonoids in the extract.

Estimation of phenols and tannins: Ferric chloride test

Two mL of $FeCl_3$ solution was added to 1 mL of *M. indica* leaves extract. The bluish-black colour was developed, indicated the presence of phenols in the extract.

Estimation of steroid: Lieberman-Burchard's test

Three mL of chloroform was mixed with 1 mL of *M. indica* leaves extract then added 15 drops of acetic acid and 8 drops of concentrated H_2SO_4. The change of red colour from blue to green indicated the presence of steroids.

Estimation of glycosides: Keller-Killiani method

About 3 drops of 3% $FeCl_3$ solution and a few drops of glacial acetic acid were added to 1 mL of *M. indica* leaves extract. The mixture was then treated with concentrated H_2SO_4. The formation of two layers and interface with a brown ring indicated the presence of glycosides in the extract sample.

Estimation of saponins

Three mL of *M. indica* leaves extract was diluted with 7 mL of distilled water and shaken vigorously.

The appearance of stable foam indicated the presence of saponins in the extract.

Animals

Swiss albino mice (*Mus musculus*) (8-10 weeks old, and 25 g to 30 g of weight) were obtained from CSIR-CDRI (Central Drug Research Institute), Lucknow. Animals were kept in polycarbonate cages on a 12 h light and dark cycle at 22±3°C temperature and 50-60% of relative humidity. Food and water were supplied *ad libitum*. The experiments were approved by the Institutional Animal Ethics Committee (IAEC) of the University of Lucknow, Lucknow with reference No. 03/I/2023/IAEC/LU, and the study was conducted according to the CCSEA guidelines. The acute toxicity study of ethanolic extract of *M. indica* leaves was performed based on Organisation for Economic Co-operation and Development (OECD) Guideline 420 (OECD 2001)[13]. The study was divided into two parts: the sighting study; and the main study.

Sighting study

Sighting study was conducted with the purpose to minimize the number of mice to be used and to select the optimum dose to be used in the main study. Mice were fasted for 3 h (only food was withdrawn but not water) and weighed. A single oral dose of ethanolic extract of *M. indica* leaves was administered to each experimental set of mice at the rate of 50, 300, and 2000 mg kg^{-1} b.w. respectively using a gavage. After the administration of extract the food was supplied in access to animals for next 2 h.

Main study

Mice were divided into 2 groups namely control and treatment groups of five (n=5) animals each. The experimental dose of 2000 mg kg^{-1} b.w. of *M. indica* leaves extract from the sighting study was selected for the main study. After 3 h of fasting 2000 mg kg^{-1} b.w. ethanolic extract of *M. indica* leaves was administered orally once to each mouse. The vehicle (distilled water) was given to the control group.

After extract administration during the first 4 h, mice were observed individually, followed by two observations each day up to 14 days for the purpose of recording symptoms of ill-health, behavioural changes, toxicity signs and mortality. The body weight of each mouse was recorded subsequently on day- 0, 2, 4, 6, 8, 10, 12 and 14.

Gross necropsy and blood collection

On completion of toxicity studies (15^{th} day), all the animals were euthanized. A gross necropsy was performed to check any abnormalities in organs including liver, kidney, spleen, lung, heart and testes. The blood was collected into a glass tube containing anticoagulant through the cardiac puncture. Blood was centrifuged at 10,000 rpm for 10 min to obtain serum (Spinwin-MC 02 (Tarsons), Daihan Scientific Co. Ltd. Korea). The biochemical analysis of serum was performed using a colorimetric method (UV-1800, Shimadzu, Japan) for aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium and creatinine. Serum ALT and AST were determined using the method of Frei[14]; Reitman and Frankel[15]. Serum calcium was determined according to the method described by Stern and Lewis[16]. Serum creatinine was estimated by the method of Toro and Ackermann[17]. Stomach, spleen, kidney, liver, lung, heart, brain and testes were collected and absolute organ weights were taken. Relative organ weight (ROW) was calculated using the following formula[18].

$$ROW = [\text{Absolute organ weight (g)}/\text{Body weight (g)}] \times 100$$

Histological study

Liver, kidney and testes were collected from dissected animals and fixed in 10% neutral buffered formalin (NBF) for routine histopathological examination. The organs were cut into small pieces, serially processed, and paraffin (m.p. 60°C, Merck, Mumbai, India) embedded. The sections were cut into 5 µm thickness using a fully automatic rotary microtome (YSI-062, Yorco Scientific Industries Pvt. Ltd. Ghaziabad, India), differentially stained with hematoxylin and eosin (H&E) and mounted with DPX. The stained sections were examined and photographed under a light microscope (Olympus CX21i, Tokyo, Japan).

Statistical analysis

The results were expressed as means ± standard deviation (SD). Student's t-test was performed to analyze the data obtained in the acute oral toxicity experiments. The significance level was interpreted as $p<0.05$. Statistical analysis was performed using GraphPad Prism (Biomatters, Ltd. NZ and GSL Biotech, USA), version 5.

Results

Phytochemical screening

Phytochemical screening of *M. indica* leaves extract revealed the presence of alkaloids, flavonoids, phenols, steroids and tannins (Table 1).

Sighting and main study

After the administration of ethanolic extract of *M. indica* leaves, mice did not show any physical and behavioural changes in both sighting and main studies throughout the 14 days study period. No toxic effects and mortality observed in both studies (Supplementary Table S1).

Body weight

Body weight increased by more than 16.14% during the study period in both the sighting and main studies. In the main study, the mean body weight of mice was significantly linearly increased when compared with the mean body weight on day 0 (Supplementary Table S2).

Gross necropsy

Gross necropsy findings did not show any changes in the organs examined. The organ of mice did not show malignancy or lesion. The relative organ weight for the stomach, spleen, kidney, lung, heart, brain and testes of the sighting and main study did not show any differences when compared with control (Fig. 1a & b).

Biochemical analysis

Serum biochemical analysis of ALT, AST, calcium, creatinine and calcium/creatinine ratio for the sighting and main studies are shown in Table 2. The values of biochemical parameters reflect insignificant changes when compared with control.

Histology

In histological investigation of H&E stained sections of the liver, kidney and testes of mice mostly showed normal architecture at all doses of *M. indica* in the sighting and main studies. The liver of all the treated and untreated mice showed the normal morphology of hepatocytes and normal structure of the central vein and sinusoids when compared with control (Fig. 2 and Fig. 3). Lobular organization follow usual formation of hepatocyte cords radiating from a central vein. Kupffer cells (KC) normally intervenes sinusoidal walls, distributed regularly and no exceptional Kupffer cell proliferation was seen. There was no evidence of central vein necrosis and immune cell infiltration or hepatocellular necrosis or sinusoidal congestion in the liver. In main study minor hydropic changes (observe solid short white arrows indicating) were observed in histopathology of liver of mice treated with 2000 mg kg^{-1} b.w. extract

Table 1 — Phytoconstituents from *M. indica* leaves extract percolates

	Type of extract				
Phytochemical constituent	Ethanol	Methanol	Aqueous	Hexane	Chloroform
Alkaloids	+	+	-	-	-
Flavonoids	+	+	+	-	-
Phenols	+	+	+	-	-
Steroids	-	+	+	-	-
Glycosides	-	-	-	-	-
Tannins	+	+	+	-	-
Saponins	-	-	-	-	-

(+): Presence; (-): Absence

Table 2— Biochemical parameters test results of the sighting and main studies

Study	Sighting study				Main study	
Parameter	Control	50 mg kg^{-1}	300 mg kg^{-1}	2000 mg kg^{-1}	Control	2000 mg kg^{-1}
ALT (U/L)	57.69	69.23	69.23	57.69	50.11±12.71	55.38±9.65
AST (U/L)	85.50	99.75	92.63	78.38	85.50±6.30	78.38±7.13
Calcium (mg dL^{-1})	7.32	7.56	7.32	8.05	7.51±0.20	7.61±0.20
Creatinine (mg dL^{-1})	0.95	0.95	0.87	0.95	0.95±0.08	0.87±0.10
Ca/creatinine ratio (mg dL^{-1})	7.71	7.96	8.41	8.47	7.95±0.71	8.81±0.77

For the main study, the values are expressed as mean ± standard deviation, n = 5. ALT; alanine aminotransferase, AST; aspartate aminotransferase. Ca; calcium.

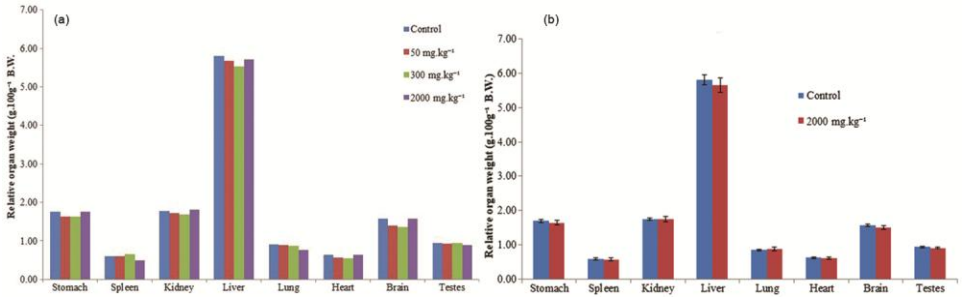

Fig. 1 — The relative organ weight of mice treated with *M. indica* leaves extract from the sighting (a) and main studies (b)

(Fig. 3-b, -c, -e) as compared with control. However, some of treated mice were showing no such hydropic changes indicating minor hydropic changes are reversible. Binucleated hepatocytes represent the major dividing cell type in the regenerating liver[19], but unusual numbers of binucleated hepatocytes were not observed in comparison to control. The histopathological assessment of the kidney of all treated mice, even 2000 mg kg^{-1} b.w. of leaf extract, showed no change in the architecture of glomerular and renal tubules. No degenerative changes in the renal tubule epithelial lining were found. No signs of tubular atrophy or acute tubular necrosis were observed (Fig. 4 and Fig. 5). Moreover, in main study no congestion in kidney tubules and no renal arterioles hyperaemia were observed in histopathology of kidney of mice treated with 2000 mg kg^{-1} b.w. extract (Fig. 5). The histopathological examination of the testes showed normal architecture of seminiferous tubules with regular basement membranes (Fig. 6 and Fig. 7). Moreover, no reduction or absence of germ cells in seminiferous tubules was observed in histopathology of testes of mice treated with 2000 mg kg^{-1} b.w. extract. No Interstitial Edema or Fibrosis observed. There was no sign of inflammation and immune cell infiltration (Fig. 7).

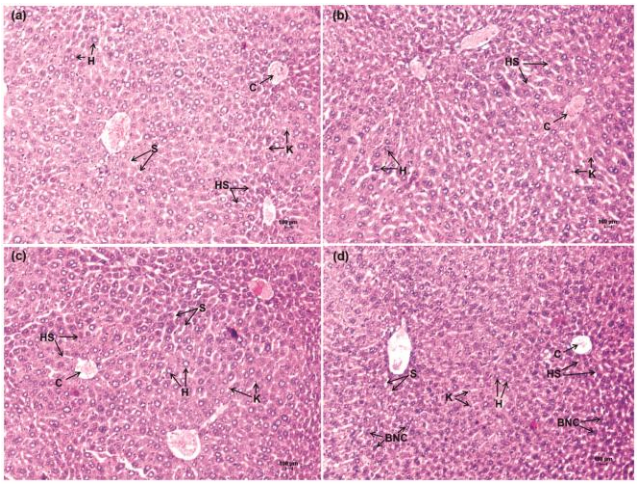

Fig. 2 — Histology of the liver of mice from the sighting study. a- Control mice, b- 50 mg kg^{-1} b.w. treated mice, c- 300 mg kg^{-1} b.w. treated mice, d- 2000 mg kg^{-1} b.w. treated mice. C: central vein, S: sinusoids, H: hepatocytes, HS: hepatic strands, K: Kupffer cells, BNC: binucleated hepatocytes. H&E Stain; 100X

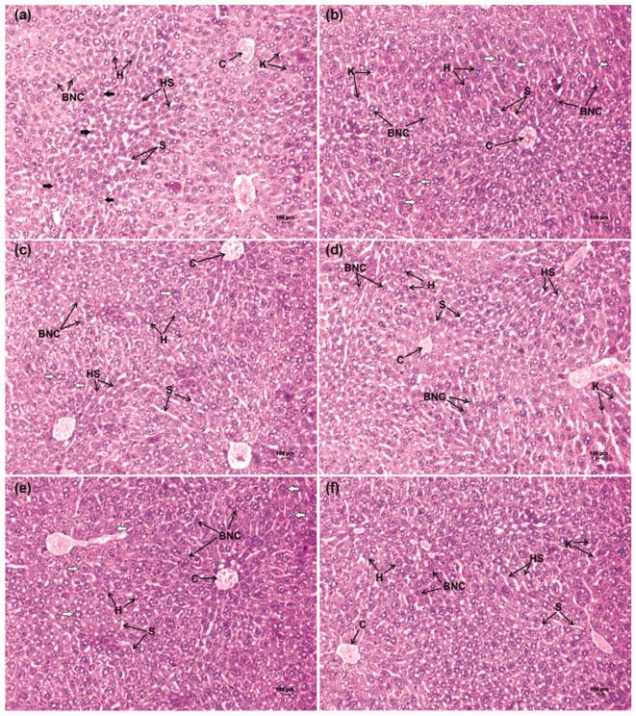

Fig. 3 — Histology of the liver of mice from the main study. a- Control, b to f- 2000 mg kg^{-1} b.w. treated mice. C: central vein, S: sinusoids, S: sinusoidal proximity, H: hepatocytes, HS: hepatic strands, K: Kupffer cells, BNC: binucleated hepatocytes; observe solid short white arrows indicating minor hydropic changes in hepatocytes (2b, 2c, 2e) as compared with control (2a, observe short black arrows indicating normal hepatocytes). H&E Stain; 100X

Discussion

Ayurveda is one of the traditional systems of medicine that practices person-centred medicine (PCM), which deals with healthy lifestyle, health promotion and sustenance, disease prevention,

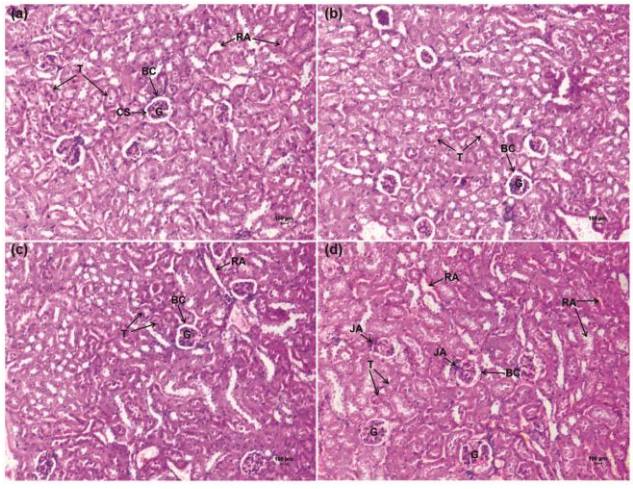

Fig. 4 — Histology of kidney of mice from the sighting study. a- Control mice, b- 50 mg kg^{-1} b.w. treated mice, c- 300 mg kg^{-1} b.w. treated mice, d- 2000 mg kg^{-1} b.w. treated mice. G: glomerulus, CS: capsular space, BC: Bowman's capsule, T: renal tubules, RA: renal arteriole, JA: juxtaglomerular apparatus. H&E Stain; 100X

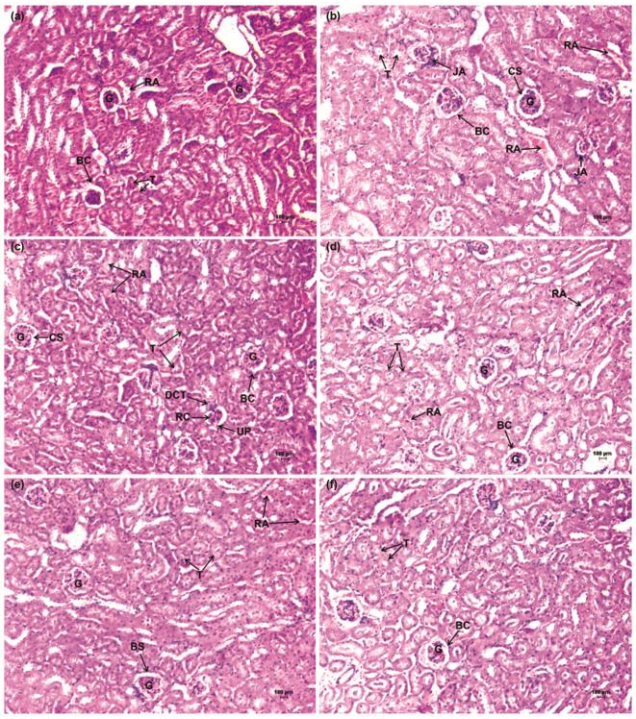

Fig. 5 — Histological analysis of kidney of mice from the main study. a- Control mice, b to f- 2000 mg kg^{-1} b.w. treated mice. G: glomerulus, CS: capsular space, BC: Bowman's capsule, T: renal tubules, RA: renal arteriole, JA: juxtaglomerular apparatus, DCT: distal convoluted tubule, RC: renal corpuscle, UP: urinary pole. H&E Stain; 100X

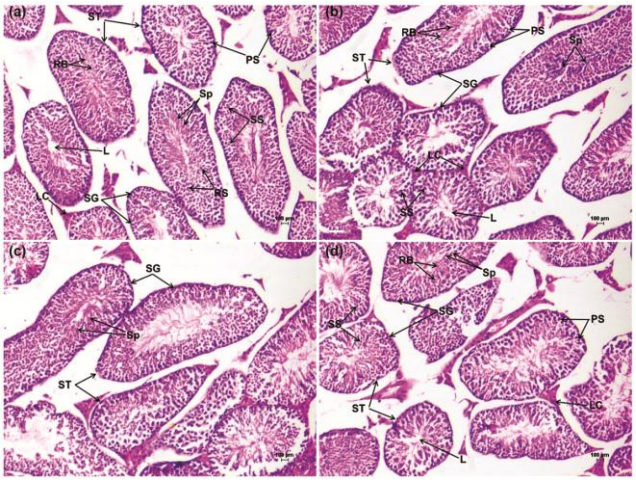

Fig. 6 — Histological analysis of testes of mice from the sighting study. a- Control mice, b- 50 mg kg^{-1} b.w. treated mice, c- 300 mg kg^{-1} b.w. treated mice, d- 2000 mg kg^{-1} b.w. treated mice. L: Lumen, LC: Leydig cells, PS: primary spermatocytes, Sp: spermatozoa, SG: spermatogonium, SS: secondary spermatocytes, ST: seminiferous tubule, RB: residual bodies, RS: round spermatids. H&E Stain; 100X

diagnosis and treatment. Herbal medicines offer a cheaper, holistic, safer and person-centred approach

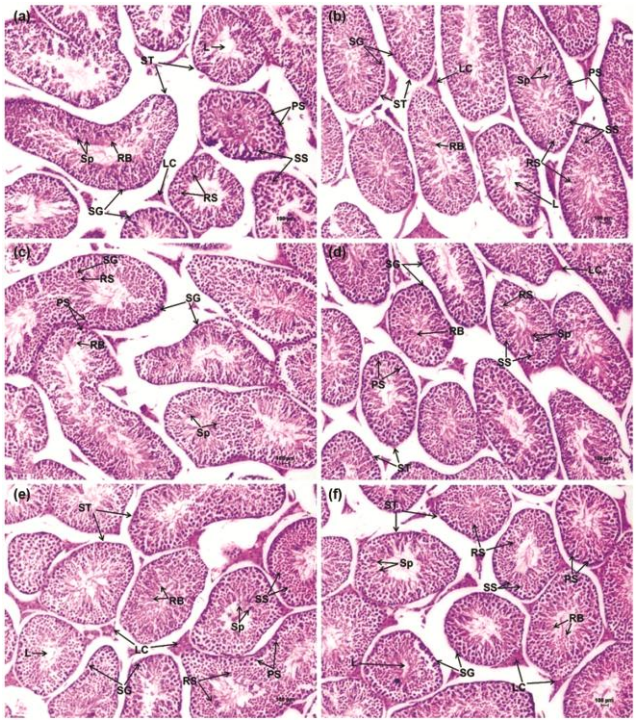

Fig. 7 — Histological analysis of testes of mice from the main study. a- Control mice, b to f- 2000 mg kg^{-1} b.w. treated mice. L: Lumen, LC: Leydig cells, PS: primary spermatocytes, Sp: spermatozoa, SG: spermatogonium, SS: secondary spermatocytes, ST: seminiferous tubule, RB: residual bodies, RS: round spermatids. H&E Stain; 100X

for the treatment of various diseases and severe metabolic conditions. Such medicines are accepted for their social aspects and feasible for economic importance[20]. However, studies have revealed that many therapeutic plants have negative side effects[21], raising concerns about the possible harmful implications of their usage. Therefore, it is essential to assess the toxicity patterns of every medicinal plant extract intended for clinical use in humans to determine how safe it is to consume[22].

Toxicity studies provide a platform for evaluating safety and identifying hazards[23]. Adverse effects that manifest immediately or quickly, often within 24 h of oral intake of the test chemical are referred to as acute oral toxicity[24,25].

The differences in body weight might be influenced by numerous factors, including alterations in growth hormone or somatostatin that can be changes in growth, alterations of hormonal consequences that can be seen in the secretion of sex steroids and alterations in neurotransmitters that can affect the consumption of food[26]. In addition, the environmental factors that can also cause stress to the

animals and result in body weight changes. However, in this study, the body weight was increased linearly in all mice with more than 16% change from day 0 to 14 days demonstrating the normal growth of mice with no sign of toxicity.

An effective sensitive indication of chemically induced alterations to organs has been shown to be variations in organ weight. The liver is the most vital organ for toxicity studies because of its involvement in metabolising and removing chemicals from the body[27]. In both of sighting and main studies, the relative organ weight of each organ exhibited no malignancies or abnormalities, and no significant differences were observed with the highest dose of 2000 mg kg^{-1} b.w. of ethanolic extract of *M. indica* leaves used in this study.

Enzymes are considered indicators because cytotoxic effects of a compound are easily ascertained by estimating the altered enzymatic activity in the cells[28,29]. Enzyme AST can be found in various tissues, like the liver, kidney, heart, lungs, brain, pancreas, skeletal muscle, erythrocytes and leukocytes. This enzyme is prominent for cardiac and muscle disease. Fatty liver and non-alcoholic steatohepatitis can also be seen due to mild elevation of AST; acute or chronic hepatitis can be induced by moderate elevation of AST; Severe viral hepatitis, drug-induced hepatic necrosis can be induced due to high AST altitude[30]. Additionally, because the AST enzyme is not as sensitive or specific to the liver, its increase may also be seen because of non-hepatic factors[31]. However, there were no notable changes in relative liver weight. There were no malignant hepatic lesions. Liver histology revealed no signs of hepatocellular necrosis, or inflammatory infiltration.

ALT is the biochemical marker for liver toxicity since it is largely located in the liver, making it liver specific. ALT levels that are greater than AST levels in the plasma suggest liver toxicity because of the leaking of damaged or necrotic cells[32,33]. The current study revealed no rise in ALT levels, and the values are comparable to control, implying that no liver damage occurred. These findings were supported by no apparent changes in relative liver weight and no aberrant findings on liver histology in any mice treated with varied doses of *M. indica*.

Toxicity may lead to damage of the integrity of the cell membrane of kidney resulting in cellular leakage of certain biomarkers and loss of function. Higher creatinine level is one of the outcomes of reduced glomerular filtration rate (GFR) which demonstrate the decreased efficiency of kidney for excreting waste products[34]. In addition, reduced GFR along with impaired renal function and acute kidney injury may lead to elevated serum calcium level[35]. Calcium homeostasis is regulated by coordinated action of parathyroid hormone, calcitonin and vitamin D metabolites. The increased level of calcium and creatinine ratio revealed the physiological alteration caused by toxicity which may cause development of nephrolithiasis[36]. The current study demonstrated no significant alteration in calcium, creatinine and their ratio, therefore we can conclude that 2000 mg kg^{-1} body weight of *M. indica* leaves extract did not cause any toxicity.

Histopathological analyses are crucial for safety assessment during the intake of certain substances since they validate the findings in hematological and biochemical analysis[37]. The current study performed a comprehensive histopathological analysis of the kidney, liver and testes of all tested animals.

Craig *et al.*, investigated the relationship between the renal histopathological changes with chemically induced changes in absolute and relative kidney weight. Their finding suggests that the change in absolute kidney weight was beneficial in recognizing potential renal toxicants when correlated with accompanying histopathological alterations after exposure with a chemical[38]. In the current study, no histopathological changes were observed in kidneys in animals treated with high dose of 2000 mg kg^{-1} b.w. ethanolic extract of *M. indica* leaves. In the cross-sections of the kidney, the glomeruli, distal convoluted tubules (DCT), proximal convoluted tubules (PCT) and Bowman's space appeared to be normal. In renal cells no interstitial and intra glomerular congestion, tubular atrophies, degeneration, bleeding or necrosis were observed when compared with control group. In histopathological analysis of liver, the lysosomal hydrolytic enzymes leakage might cause cytoplasmic degeneration and macromolecular crowding which may lead to hepatocellular swelling[39]. The Kupffer cells take part in removal of accumulated toxic substances by activating lysosomal enzymes resulting in breakdown of substances into small metabolic products. The hepatic toxicity induced kupffer cells hyperplasia is a defence mechanism of detoxification[40]. In current study no hepatocellular and central vein necrosis or sinusoidal congestion

were observed. The hepatocytes were clearly visible with normal blood cells, no Kupffer cells hyperplasia was observed. These results revealed the absence of hepatotoxicity in the liver. In testicular cells, normal seminiferous tubules with regular basement membrane and spermatogenic cells were observed. The interstitial spaces having Leydig cells were normally distributed.

A wide range of natural compounds having antioxidant properties is widely used to relieve oxidative stress[41]. Flavonoids, alkaloids, terpenes and phenols are responsible for the antioxidant activity found in *M. indica* leaves. Phenolic compounds are powerful antioxidants that can protect various tissues including liver and kidneys from damage caused by free radicals[41,42].

According to the finding from the sighting and main studies of the acute oral toxicity of *M. indica* extract at a dose of 2000 mg kg^{-1} b.w. did not result in acute hepatotoxicity in mice. The absence of behavioural and physical alterations, mortality and any notable variations in body weight, relative organ weight and biochemical parameters all confirm these findings. Garrido *et al.* (2009), found no deaths and gross histopathological alterations after administration of *M. indica* stem bark extract at a single oral dose of 2,000 mg kg^{-1} in male and female mice[43]. This upholds our study on acute toxicity of *M. indica* leaves extract.

Hazard assessment criteria category 5 are intended to facilitate the identification of test compounds that are of low acute toxicity hazard. Considering the Globally Harmonised System (GHS) classification, *M. indica* might be labelled as unclassified (category 5)[13].

Conclusion

According to OECD Guideline 420, a test item can be categorized as category 5 or unclassified under the GHS category if there is no indication of toxicity up to 2000 mg. kg^{-1} b.w. in the main study. However, due to the lack of any identifiable target organ or evidence of systemic toxicity, *M. indica* up to 2000 mg. kg^{-1} b.w. can be categorized as unclassified under GHS classification. Therefore, findings of this study suggest that oral administration of *M. indica* extract up to 2000 mg. kg^{-1} b.w. results in no toxicity.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_24(1)(2025)23-32_SupplData.pdf

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

R.C. contributed to conception, resources, design and writing. R.G. visualized the figures and tables and elaborated the discussion part. S.S. contributed to review of literature, data analysis and writing. P.Y. conducted literature surveys and arranged the references. A.M.S. reviewed and edited the manuscript. M.G. supervised, co-wrote and edited the manuscript. All authors reviewed the manuscript and gave consent to publish it.

Ethics Approval

The experimental animals were approved by the Institutional Animal Ethics Committee (IAEC) of the University of Lucknow, Lucknow, approval no. 03/I/2023/IAEC/LU, and the study was conducted according to the Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines.

Data Availability

The authors confirm that all the data supporting the findings of this study are available within the article.

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