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RESEARCH ARTICLE

Safety Evaluation of Novel Siddha Herbal Formulation Maramanjal Chooranam: Acute and Subacute Toxicity Evaluations in Sprague Dawley Rats

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Abstract

Background: Maramanjal Chooranam (MMC) is a novel siddha herbal medicine believed to offer therapeutic benefits such as anti-inflammatory and hepatoprotective effects. Evaluating the toxicological effects associated with herbal medicine is a crucial public health concern that must be addressed to better establish its clinical efficacy. **Aim and Objective:** Aim of the present study is to evaluate the acute and sub acute toxicity of MMC in Sprague Dawley rats, through biochemical, hematological, and histopathological analyses. **Materials & Methods:** The acute toxicity study involved administering a single oral dose of 2000 mg/kg of MMC to Sprague Dawley rats (weighing 180 $\pm$ 200 g, aged 8–10 weeks). The rats were observed for 14 days for any signs of toxicity, and their body weights were recorded regularly. Additionally, a sub-acute toxicity study was conducted on Sprague Dawley rats with oral doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg of body weight. After euthanasia, blood samples were taken for biochemical and hematological analysis, and vital organs were examined histopathologically. **Results:** No mortality was witnessed after 14 days of observation period. Behavioral observations showed normal. Subacute toxicity results indicated no significant adverse effects on body weight or organ weights across the MMC-treated groups. Biochemical analyses revealed no significant change in liver enzymes and lipid parameters. Hematological evaluations showed normal values. Histopathological examination demonstrated normal tissue architecture in all major organs with no significant pathological findings. **Conclusion:** From the study it was concluded that MMC did not cause any mortality or behavioural changes at a single dose of 2000 mg/kg and seems relatively safe at the tested doses for a 28-day period, with no severe systemic toxicity.

Keywords: Acute toxicity study; Subacute acute toxicity study; Histopathological evaluation; OECD 423; OECD 407; Maramanjal Chooranam; Siddha

1 Introduction

For centuries, plants have been utilized in the Siddha system for a multitude of purposes, particularly as food and medicine for nutrition and disease treatment. They have been relied upon for millennia to support, promote, and restore health. The use of herbal medicines is driven by various factors, including their accessibility, affordability, and minimal adverse effects. Plants synthesize a range of metabolites, some beneficial and others potentially toxic to humans[(1)]. The selective uptake or accumulation of specific xenobiotics in particular tissues or cells, the inhibition of normal export of potentially toxic metabolites, and the activation of cellular receptors can lead to toxicity[(2)].

Toxicity studies on herbal medicines should mirror their traditional use to facilitate rational discussions regarding their safety and benefits. Despite many medicinal herbs being natural and processed physically and/or biologically, there is limited scientific data available on their safety[(3)]. Additionally, pharmaceutical drugs can be therapeutic at one dose and toxic at another[(4)].

According to OECD guidelines, such as 401, 407, 423, and 425, medicines cannot be used in therapeutic settings without undergoing clinical studies and toxicity testing[(5)]. Acute and sub-acute toxicity tests are routinely conducted to evaluate the safety of any drug. The acute toxicity study is the initial step to determine the adverse effects of a substance within 14 days following a single oral administration[(6)]. As per OECD 407, in a sub-acute toxicity study, a drug is administered daily, usually for 28 days, to rodents (rats), dogs, or monkeys[(7)]. The drug can be administered to three groups of animals at three different doses.

The highest dose level is chosen to induce toxicity without causing death or severe suffering. The middle dose is set to induce clear but mild toxicity, demonstrating a dosage-related response, while the low dose is chosen to demonstrate a no-observed-adverse-effect level (NOAEL)[(8)]. The liver and kidneys, being primary organs affected by metabolic reactions of toxicants, are critical in predicting toxicity effects of phytotherapeutic products or drugs[(9)]. In experimental animals, liver function tests help understand toxic effects, which can be extrapolated for human safety[(10)]. Blood parameters are key indicators of potential health hazards and have a high predictive value for toxicity[(11)]. Calculated indices are used to define anemia, hemoglobin concentration, platelet volume, and count[(12)]. These blood parameters help determine toxicity in relation to dose and time-response[(13)].

Maramanjal Chooranam (MMC) is a traditional Siddha formulation composed of three herbal ingredients, used for various therapeutic purposes such as treating fever, inflammation, and liver disorders. Despite its lengthy history of usage in traditional medicine, there is a considerable void in scientific data regarding MMC's overall toxicity profile. One obstacle to its broader adoption and incorporation into contemporary medical procedures is the lack of comprehensive toxicological data. To Address this gap in this study, we aimed to conduct systematic evaluation of acute and sub-acute toxicity profiles in Sprague Dawley rats providing necessary scientific evidence to ensure the safety and therapeutic use of the Siddha herbal formulation, Maramanjal Chooranam.

2 Methodology

2.1 Identification and collection of Plant materials

The ingredients of maramanjal churnam are:

- *Coscinium fenestratum*
- *Toddalia asiatica*
- *Picrorhiza kurroa*

The plant materials were gathered from natural habitats in and around the Thenkasi district, Tamil Nadu, between August and December. Dr. Sankaranarayanan identified the plants, and the specimens were submitted to the Herbarium at the Government Siddha Medical College in Arumbakkam, Chennai and got the authentication number- M01112204C. The specimens were then authenticated taxonomically, and corresponding collection numbers were assigned.

Medicinal plants used in the study: The stem of *Coscinium fenestratum*, known locally as Maramanjal and belonging to the Menispermaceae family, is used in the form of powder and decoction to treat indigestion, flatulence, liver diseases, and fever. Additionally, a decoction of its bark is used in treating intermittent fevers[14].

Toddalia asiatica, from the Rutaceae family and locally called Milagaranai, employs its root and leaves for various treatments. It is used to treat nasal and bronchial pains, snake bites[15], It has anti-inflammatory, analgesic and cardioprotective actions[16].

Picrorhiza kurroa, belonging to the Plantaginaceae family and known as Katuki, uses its root and rhizome for medicinal purposes. It is utilized to treat dyspepsia and hepatitis[17] and serves as a purgative, brain tonic, stomachic, and antiperiodic[18].

2.1.1 Preparation of herbal formulation

- Stem of *Coscinium fenestratum* - 2 part
- Root bark of *Toddalia asiatica* - 1 part
- Root of *Picrorhiza kurroa* - 1 part
- All the parts were dried under shade and ground to fine powder.
- Dosage: 1gm (BD)
- Adjuvant: Hot water
- Indication: Putru Noi (Cancer)

2.2 Experimental animals

The study was carried out at experimental animal facility, KK College of Pharmacy, Chennai, (8550/KKCP/2020). Sprague Dawley rats, weighing between 180 and 200 grams and aged 8–10 weeks, were randomly selected for the study. They were housed under controlled conditions, maintaining a room temperature of 22 $\pm$ 2°C, a constant humidity of 55 $\pm$ 5%, adequate ventilation, and a 12-hour light/dark cycle. The rats were fed a standard diet of pellets daily and had unlimited access to tap water. They were acclimatized to these standard laboratory conditions for seven days prior to the commencement of the experiment.

The toxicity assays were conducted in accordance with the guidelines established by the Organization for Economic Cooperation and Development (OECD, 2001) for the use of animals in scientific research. The animal experiments were carried out at the Laboratory of Pharmacology, K. K. College of Pharmacy, Chennai, with the necessary ethical approval. All procedures adhered to the guidelines set forth by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA).

2.3 Experimental design for acute toxicity[19]

In accordance with OECD Test Guidelines 423, nulliparous and non-pregnant female Sprague Dawley rats, weighing between 180 and 200 grams and aged 8–10 weeks, were randomly selected for the study. A single oral dose of 2000 mg/kg of MMC was administered. The rats were fasted for 3–4 hours before dosing but had free access to water. Post-dosing, the animals were closely observed for the first 30 minutes and then for 4 hours. Food was reintroduced 1–2 hours after dosing. The rats were monitored for any signs of toxicity within the first 6 hours and at regular intervals over a 14-day period. The surviving rats were further observed for signs of toxic reactions, such as rising fur, draping, tremors, excitability, twitching, salivation, and mortality, until the end of the 14-day period (OECD). After 14 days, the rats were humanely sacrificed.

2.4 Experimental design for 28 Days repeated dose oral toxicity (sub-acute toxicity)[8]

The repeated oral dose 28-day (sub-acute) toxicity study was conducted following OECD Guideline No. 407. Sprague Dawley rats, both male and female, weighing between 180 and 200 grams and aged 8–10 weeks were selected for the study. The animals were divided into three groups: Group I received a low dose of MMC, Group II received an intermediate dose, and Group III received a high dose. Each group consisted of five rats of each gender. The respective doses of 200 mg/kg, 400 mg/kg, and 800

mg/kg were administered orally under controlled conditions for 28 consecutive days. Group I served as the low dose group, Group II as the intermediate dose group, and Group III as the high dose group.

2.4.1 Body weight measurements

Using a Mettler PE 1600 analytical balance (+0.01 g, Switzerland), the weight of the control and experimental rats was measured on day 1 (before the test extracts were administered), on day 7, and on day 14 to assess acute toxicity. In subacute toxicity study, the weight was recorded almost daily till day 28.

2.4.2 Hematological studies

After 28 days of repeated oral administration of Maramanjal Chooranam (MMC), the rats were anesthetized using inhalation of Isoflurane (C3H2ClF5O, Forane) which is followed by gentle cervical dislocation. After that, a high concentration (3%) of anesthetic gas was injected into the sealed glass chamber where the euthanized rats were kept until they died peacefully due to central nervous system depression. Each rat's blood was drawn and placed in test tubes with and without EDTA. After allowing the EDTA-free blood samples to clot, the serum was separated using centrifugation for 20 minutes at 2000 rpm and 4°C. Hematocrit, hemoglobin concentrations, erythrocyte count, reticulocytes, total and differential leukocyte count, platelet count, and a measure of blood clotting time/potential were all assessed at the conclusion of the test period.

2.4.3 Estimation of biochemical markers

The concentrations of urea, creatinine, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein were assessed using a Semi-Autoanalyzer equipped with Tranasia and Erba kits.

2.4.4 Histopathological examinations

During necropsy, internal organs were examined to detect any obvious abnormal changes. The vital organs of each animal were weighed. Subsequently, the organs were processed histopathologically and placed in 10% buffered formalin. The specimens were sectioned at a thickness of 5 μm, embedded in paraffin wax, trimmed, and stained with hematoxylin and eosin. These sections were then examined under a light microscope, and magnified images of the tissue structures were captured for further analysis.

2.5 Statistical analysis

The statistical analysis of the animal experiments was conducted using two-way ANOVA with GraphPad Prism, version 9.4 for Windows, followed by Tukey's post hoc t-test. Data are expressed as the mean $\pm$ standard error of the mean (Mean $\pm$ SEM). Results from the study groups were compared with the control group, with significance levels set at $p < 0.05$, $p < 0.01$, and $p < 0.001$, indicating statistically significant, highly significant, and very highly significant differences, respectively.

3 Results

3.1 Acute Toxicity Study

Following drug administration, systematic observations and records were made in accordance with the guidelines. MMC at a dosage of 2000 mg showed no signs of mortality.

3.1.1 Behavioural Pattern

During the acute toxicity study, no notable behavioral alterations were seen as shown in Table 1.

Table 1. Effect of MMC on behavioral pattern of rats in acute toxicity study

Parameters/Time	30 Min		4 Hrs		24 Hrs		48Hrs		7^{th} Day		14^{th} Day	
	C	MMC	C	MMC	C	MMC	C	MMC	C	MMC	C	MMC
Fur and skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
respiration	N	N	N	N	N	N	N	N	N	N	N	N

Continued on next page

Table 1 continued

Urination (color	N	N	N	N	N	N	N	N	N	N	N	N
Feces consistency	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions and tremors NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Tremors	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Itching	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

C = Normal control group, MMC = Maramanjal Choornam, N = Normal, NF = Not found

3.1.2 Organ to Body Weight Ratio:

No lesions were observed during the examination of the isolated organs from the test animals. The organ-to-body weight index was calculated and is summarized in Table 2, indicating that there were no significant variations among the groups.

Table 2. Effects on organ to body weight indices

Organs	C	MMC
Brain	1.453 ± 0.042	1.501 ± 0.034
Spleen	0.423 ± 0.012	0.437 ± 0.009
Heart	0.761 ± 0.022	0.786 ± 0.017
Lung	1.138 ± 0.033	1.175 ± 0.026
Liver	5.152 ± 0.151	5.320 ± 0.121
Kidney	1.461 ± 0.043	1.509 ± 0.034
Adrenal	0.062 ± 0.001	0.064 ± 0.001
Ovary	0.069 ± 0.002	0.071 ± 0.001

C = Normal control group, MMC = Maramanjal Choornam, Values are presented as mean ± SEM; N = 3

3.2 Sub-acute Toxicity Study

From the results, it was observed that no significant change was observed in the body weight of Sprague-Dawley rats when compared to control group.

Table 3. Body Weight of rats in control group and MMC treated group

Days	Control	MMC (200mg/kg)	MMC (400mg/kg)	MMC (800mg/kg)
Day 0	185.4 ± 2.83	184.6 ± 4.06	179.5 ± 7.51	174 ± 8.52
Day 4	188.4 ± 2.39	189.3 ± 6.11	182.8 ± 7.53	177.6 ± 8.72
Day 8	191.7 ± 2.34	194.7 ± 7.62	186.8 ± 6.97	181.4 ± 8.73
Day 12	192.9 ± 7.53	199 ± 8.67	190.7 ± 7.11	185.1 ± 8.68
Day 16	196.4 ± 7.06	203 ± 8.93***	194.4 ± 6.88***	188.7 ± 9.09
Day 20	200.1 ± 6.76*	207.3 ± 9.72***	198.2 ± 7.15***	192.5 ± 9.23***
Day 24	205.7 ± 7.39***	212 ± 10.39***	202.8 ± 6.95***	196.3 ± 9.69***
Day 28	210.1 ± 7.61***	215.6 ± 10.64***	206.9 ± 6.56***	200.1 ± 9.41***

Values are expressed as Mean ± SEM, n = 10, *p < 0.05 ***p < 0.001

Table 4. Hematological parameters of rats in control group and MMC treated group

	Units	Control	MMC (400mg/kg)	MMC (800mg/kg)
G	%	11.68 ± 0.41	13.12 ± 0.53***	13.43 ± 0.27***
RBC	10^6cells/μL	7.43 ± 0.13	7.66 ± 0.59	7.94 ± 0.44*@
WBC	10^3cells/μL	7.23 ± 0.34	8.08 ± 0.09***	8.10 ± 0.09***
Neutrophils	%	27.10 ± 1.85	28.53 ± 3.18	24.09 ± 3.6@@#
Lymphocytes	%	64.6 ± 2.47	65.82 ± 2.83	67.8 ± 2.28@@
Monocytes	%	2.45 ± 0.10	3.35 ± 0.21***	3.43 ± 0.26***
Eosinophils	%	1.28 ± 0.06	1.33 ± 0.12@	1.35 ± 0.05
HCT	%	49.01 ± 2.84	50.10 ± 0.91	52.02 ± 4.67
MCV	fL	60.32 ± 3.49	59.10 ± 1.55@@	61.9 ± 4.6
MCH	Pg	15.43 ± 1.38	16.05 ± 0.46	17.27 ± 0.62***#
MCHC	g/dl	26.38 ± 1.34	27.35 ± 3.07	26.68 ± 3.01
Platelets	10^3cells/μL	727 ± 43.1	706.8 ± 50.8	719.5 ± 58.9

Values are expressed as Mean ± SEM, n = 10, *p < 0.05, ***p < 0.001 (Comparison with control). @p < 0.05, @@p < 0.01

Table 5. Relative organ weights of rats in control group and MMC treated group

Organ	Control	MMC (200mg/kg)	MMC (400mg/kg)	MMC (800mg/kg)
Brain	1.523 ± 0.089	1.664 ± 0.085	1.503 ± 0.089	1.537 ± 0.055
Spleen	0.442 ± 0.026	0.484 ± 0.024	0.437 ± 0.026	0.447 ± 0.016
Heart	0.796 ± 0.04	0.871 ± 0.044	0.787 ± 0.046	0.805 ± 0.029
Lung	1.19 ± 0.069	1.303 ± 0.067	1.177 ± 0.070	1.204 ± 0.043
Liver	5.38 ± 0.316	5.899 ± 0.303	5.328 ± 0.317	5.451 ± 0.196
Kidney	1.528 ± 0.089	1.673 ± 0.086	1.511 ± 0.090	1.545 ± 0.055
Adrenal	0.064 ± 0.003	0.070 ± 0.003	0.063 ± 0.003	0.065 ± 0.002
Testis	2.566 ± 0.15	2.809 ± 0.144	2.536 ± 0.1511	2.595 ± 0.093
Ovary	0.072 ± 0.004	0.079 ± 0.004	0.071 ± 0.004	0.073 ± 0.002

Values are expressed as Mean ± SEM, n = 10, *p < 0.05 Organ weights represented in grams.

Histopathological Reports on MMC For Subacute Toxicity Study

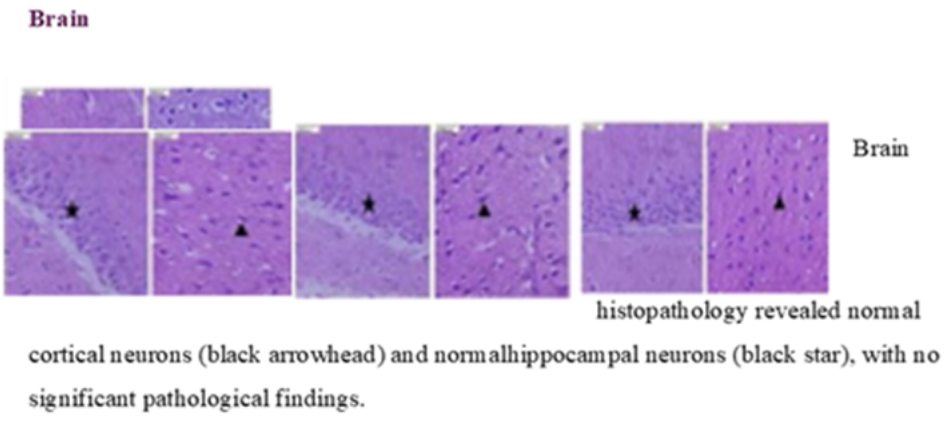


Fig 1. Histopathological Reports on MMC For Subacute Toxicity Study of Brain

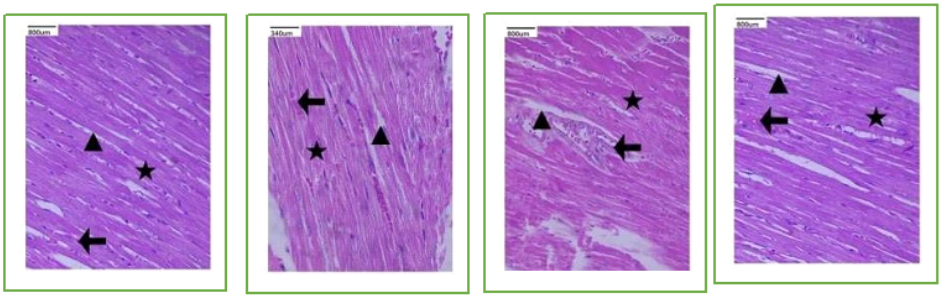
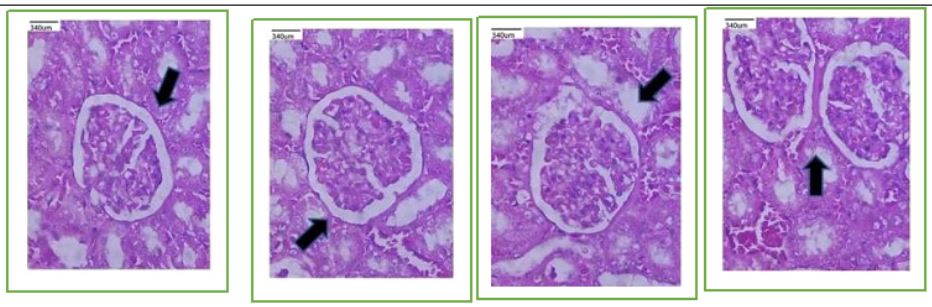
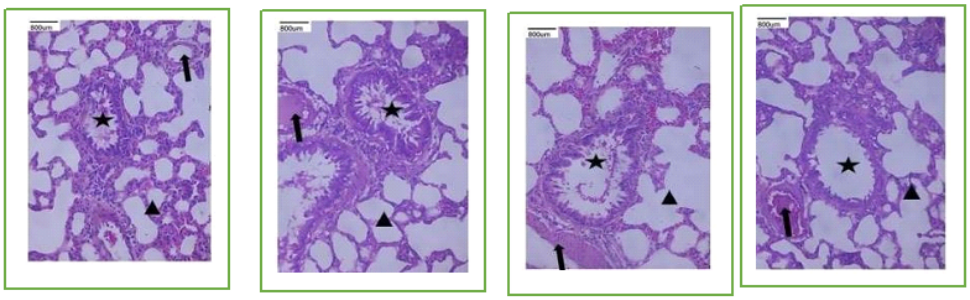
Heart	 Histopathology revealed normal cardiomyocytes (star), intermuscular connective tissue spaces (arrowhead), and blood vessels (arrow).
Kidney	 Histopathology showed normal glomeruli (arrow) with no signs of edema or inflammation. The proximal and distal convoluted tubules appeared normal, with no evidence of inflammation, edema, or apoptosis.
Lung	 Lung histopathology revealed normal alveolar structures (arrowhead), bronchioles (star), andinterstitial blood vessels (arrow). No significant pathology was observed.

Fig 2. Histopathological reports on MMC for subacute toxicity study of brain

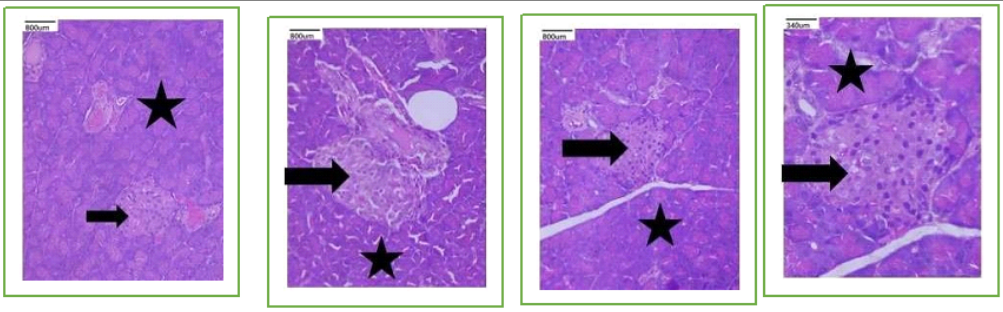
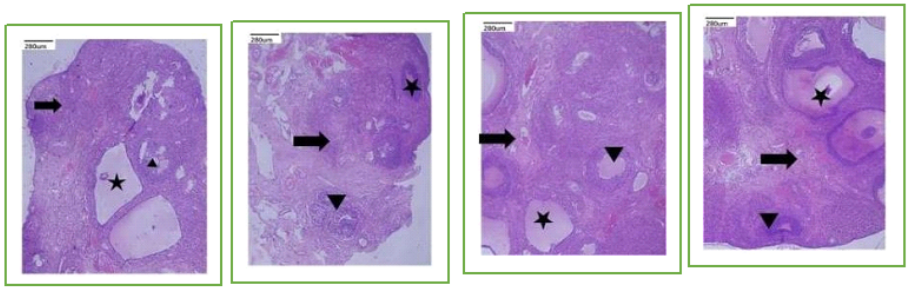
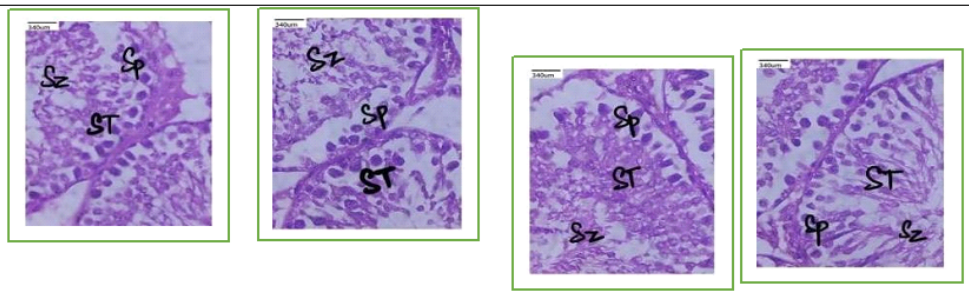
Pancreas	 Histopathology of the pancreas revealed normal islet cells of Langerhans (arrow) and normal exocrine pancreatic glands (star), with no significant pathology noted.
Ovary	 Ovarian histopathology showed normal preantral follicles (star), antral follicles (arrowhead), andnormal ovarian stroma (arrow), with no significant pathology observed.
Testis	 Histopathology of the testis showed normal seminiferous tubules (ST), spermatogonia (Sp), and spermatozoa (Sz).

Fig 3. Histopathological Reports on MMC For Subacute Toxicity Study of Pancreas, Ovary, Testis

4 Discussion

As in few instances, herbal formulations were found to be harmful, the AYUSH ministry has mandated that the safety profile of herbal formulations be assessed. For the pharmacological assessment of herbal compounds, toxicity studies are important [20]. The results of the study afford better understandings into the safety profile of MMC while administered in a single dose of MMC i.e., 2000 mg/kg body weight and at 3 different doses given repeatedly for 28 days. During the 14-days observation period, no mortality was recorded in the rats treated with MMC. The change in the body weight observed in the MMC treated animals is progressive and similar to that of the control which suggests that MMC did not adversely affect growth or induce significant toxicity at the administered dose. Behavioral observations indicated normal respiratory and somatomotor activities, with no signs of drowsiness, convulsions, or other adverse effects within the first 48 hours, indicating that MMC does not produce acute behavioral toxicity. The organ-to-body weight ratios revealed no significant changes between the MMC-treated group and the control group, suggesting the absence of organ-specific toxicity.

Body weight is a sign of health status of internal organs of the animals. MMC did not hinder the growth of Sprague-Dawley rats, as evidenced by the progressive increase in bodyweight across all groups, suggesting that MMC does not negatively impact weight gain or induce systemic toxicity, even at higher doses. Renal markers remained unaffected, suggesting no significant renal toxicity. Liver enzymes and lipid profiles showed no significant changes. MMC treatment led to an increase in hemoglobin concentration, possibly indicating an erythropoietic effect, and an increase in WBC count, particularly neutrophils and lymphocytes, suggesting an immune-modulatory response. No significant changes were observed in relative organ weights in MMC-treated groups, indicating that MMC did not cause organ hypertrophy or atrophy, aligning with the normal growth patterns and stable biochemical parameters observed.

No significant lesions remained noted in the histologiocal section of all the observed organs. A siddha formulation mandoora chooranam in which maramanjal was one of the key ingredients, also did not produce any toxic changes in wistar albino rats in both acute and sub acute toxicity evaluation [21]. Even at the highest dose levels (2000 mg/kg), acute oral toxicity of all herbal formulations did not result in any fatalities. A toxicity study helps analyze the substance's nature, whether it's toxic or non-toxic [22]. As our study drug MMC possess only herbal ingredients it seems to be safe and did not produce any significant toxicity in treated animals in both acute and subacute (28 repeated dose) toxicity studies.

5 Conclusion

The results of this analysis provide necessary data on the Acute and subacute Toxicity profile of MMC in Sprague dawley rats and this is the first data recorded on toxicity evaluation of MMC. During acute toxicity study no mortality had been identified at the dose level of 2000 mg/kg. From the results of Sub acute toxicity study, it is evident that MMC exhibited no toxic effects on the body weight and relative organ weights, hematological, biochemical, and histo pathological parameters of rats upto 800 mg/kg.

Thus, MMC is safe up to a dose range of 800 mg/kg. These findings recommend that MMC is relatively safe at the studied dosages in the context of subacute exposure, though further studies may be necessary to discover long-term effects of MMC. Future studies should concentrate on determining which active ingredients in MMC contribute to its therapeutic advantages. To confirm these results in human subjects and assure the safe and effective application of MMC in contemporary medical practice, clinical trials are advised. With these results, we can more successfully incorporate conventional Siddha formulation, MMC, into modern healthcare, offering secure and efficient remedies for a variety of illnesses.

References

1) Kale OE, Awodele O, Akindele AJ. Subacute and subchronic oral toxicity assessments of Acridocarpus smeathmannii (DC.) Guill. & Perr. root in Wistar rats. *Toxicology Reports*. 2019;6:161–175. Available from: https://doi.org/10.1016/j.toxrep.2019.01.005.
2) Sahu RK, Sahu AK. Herbal Medicines and Pharmacognosy: Exploring Efficacy and Safety. *Journal of Cardiovascular Disease Research*. 2020;11(11):89–95. Available from: https://jcdronline.org/admin/Uploads/Files/66506795551005.81465868.pdf.
3) Valdivia-Correa B, Gómez-Gutiérrez C, Uribe M, Méndez-Sánchez N. Herbal Medicine in Mexico: A Cause of Hepatotoxicity. A Critical Review. *International Journal of Molecular Sciences*. 2016;17(2):1–10. Available from: https://doi.org/10.3390/ijms17020235.
4) Sharif HB, Mukhtar MD, Mustapha Y, Baba G, Lawal AO. Acute and Subchronic Toxicity Profile of Euphorbia pulcherrima Methanol Extract on Wistar Albino Rats. *Advances in Pharmaceutics*. 2015;2015:1–9. Available from: https://doi.org/10.1155/2015/539646.
5) 5.General guidelines for safety/toxicity evaluation of Ayurvedic formulations. First edition . 2018. Available from: https://www.ayush.gov.in/docs/guideline_safety_toxicity.pdf.
6) Tauheed AM, Mamman M, Ahmed A, Sani NA, Suleiman MM, Sulaiman MH, et al. Acute, Sub-acute, Sub-chronic and Chronic Toxicity Studies of Four Important Nigerian Ethnomedicinal Plants in Rats. *Clinical Phytoscience*. 2021;7:1–12. Available from: https://doi.org/10.1186/s40816-020-00244-2.
7) Singh AK, Attrey DP, Deep PR, Dubey SU, Naved TA, Roy BA. Acute and sub-acute toxicity studies of pharmacologically active seabuckthorn leaf extract. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014;6(6):415–419. Available from: https://doi.org/10.29191/IPPS/6/2/415-419.

8) Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4. 2008. Available from: https://doi.org/10.1787/9789264070684-en.
9) Bello I, Bakkouri AS, Tabana YM, Al-Hindi B, Al-Mansoub MA, Mahmud R, et al. Acute and sub-acute toxicity evaluation of the methanolic extract of Alstonia scholaris stem bark. *Medical Sciences*. 2016;4(1):1–14. Available from: https://doi.org/10.3390/medsci4010004.
10) Ravikumar S, Gnanadesigan M. Hepatoprotective and antioxidant properties of Rhizophora mucronata mangrove plant in CCl4 intoxicated rats. *Journal of Experimental & Clinical Medicine*. 2012;4(1):66–72. Available from: https://doi.org/10.1016/j.jecm.2011.11.012.
11) Arsad SS, Esa NM, Hamzah H, Othman F. Evaluation of acute, subacute and subchronic oral toxicity of Rhaphidophora decursiva (Roxb.) Schott extract in male Sprague Dawley rats. *Journal of Medicinal Plant Research*. 2013;7(41):3030–3040. Available from: https://academicjournals.org/article/article1383308543_Arsad%20et%20al.pdf.
12) Amna OF, Nooraain H, Noriham A, Azizah AH, Husna RN. Acute and oral subacute toxicity study of ethanolic extract of Cosmos caudatus leaf in Sprague Dawley rats. *International Journal of Bioscience, Biochemistry and Bioinformatics* . 2013;3(4):301–305. Available from: https://www.ijbbb.org/papers/218-S011.pdf.
13) Elzagallaai AA, Koren G, Rieder MJ. The predictive value of the in vitro platelet toxicity assay (iPTA) for the diagnosis of hypersensitivity reactions to sulfonamides. *The Journal of Clinical Pharmacology*. 2013;53(6):626–632. Available from: https://doi.org/10.1002/jcph.85.
14) Kirubhakaran SM, Vadivelan S, Sarojini T, Mathukumar S. Anti-Diarrheal Siddha Polyherbal Formulation Maramanjal Kudineer - A Review. *International Journal of Current Research and Review*. 2021;9(7):769–773. Available from: https://ijcrt.org/papers/IJCRT2107512.pdf.
15) Wang F, Xu Y, Liu JK. New geranyloxycoumarins from Toddalia asiatica. *Journal of Asian Natural Products Research* . 2009;11(8):752–756. Available from: https://doi.org/10.1080/10286020903048975.
16) Zeng Z, Tian R, Feng J, Yang NA, Yuan L. A systematic review on traditional medicine Toddalia asiatica (L.) Lam.: Chemistry and medicinal potential. *Saudi Pharmaceutical Journal*. 2021;29(8):781–798. Available from: https://doi.org/10.1016/j.jsps.2021.05.003.
17) Raut A, Dhami-Shah H, Phadke A, Shindikar A, Udipi S, Joshi J, et al. Picrorhiza kurroa, Royle ex Benth: Traditional uses, phytopharmacology, and translational potential in therapy of fatty liver disease. *Journal of Ayurveda and Integrative Medicine*. 2023;14(1):1–9. Available from: https://doi.org/10.1016/j.jaim.2022.100558.
18) Tiwari T, Chaturvedi P. Efficient in vitro establishment and multiplication of Picrorhiza kurroa Royle ex Benth through in vitro raised seedlings. *Research on Crops*. 2016;17(4):800–807. Available from: https://doi.org/10.5958/2348-7542.2016.00135.2.
19) Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4. 2002. Available from: https://doi.org/10.1787/9789264071001-en.
20) Singh B, Sharma P, Rajput A, Singh G, Arora S. Evaluation of acute and subchronic toxicity of polyherbal formulations of Shree Dhanwantri herbals in Wistar rats. *International Journal of Pharmaceutical Sciences and Research*. 2022;13(12):5068–5079. Available from: https://doi.org/10.13040/IJPSR.0975-8232.13(12).5068-79.
21) Nanthini K, Kanakavalli K, Kaliyamurthy V. Acute and Sub Acute Toxicity Study on Siddha Drug Mandoora chooranam. *International Journal of Pharmaceutical & Biological Archives*. 2014;5(2):86–91. Available from: http://ijpba.info/index.php/ijpba/article/view/1262/898.
22) Krewski D, Andersen ME, Tyshenko MG, Krishnan K, Hartung T, Boekelheide K, et al. Toxicity testing in the 21st century: progress in the past decade and future perspectives. *Archives of Toxicology*. 2020;94(1):1–58. Available from: https://doi.org/10.1007/s00204-019-02613-4.