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# Acute and Sub-acute Toxicity Profiling of *Siddha* Formulation *Maldevi Chendhuram* in Wistar Rats

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## Abstract

**Background:** *Maldevi Chendhuram* (MC) is one of the important metallo-mineral *Siddha* formulations extensively used to treat various respiratory and nervous disorders. However, the establishment of a safety profile of MC is the need of the hour, considering its clinical importance. **Aim:** To delineate the toxicity profile of MC in Wistar rats. **Methods:** The toxicity evaluation of MC was conducted following Organisation for Economic Cooperation and Development (OECD) guidelines and included two phases. In the acute toxicity phase, MC was administered in a single dose of 50 mg/kg, 300 mg/kg, or 2000 mg/kg body weight, with a subsequent 14-day observation period. The sub-acute toxicity phase involved daily administration of MC at doses of 25 mg/kg, 125 mg/kg, and 250 mg/kg body weight for 28 days. Additionally, heavy metals were analysed in the liver tissues of control, high-dose, and satellite group animals using the AAS method. **Results:** In the acute toxicity study, no toxic signs or mortality were observed in animals at doses up to 2000 mg/kg, suggesting that the $LD_{50}$ for oral toxicity is higher than this dose. In the 28-day repeated dose oral toxicity study, no significant differences were found in body weight, food intake, water intake, haematological and biochemical parameters, or histopathological findings among the test groups, satellite group, and control group. Heavy metal analysis revealed lead at a level of 1.42±3.47 in the high-dose group, but it was not detected in the control and satellite groups. Arsenic levels were recorded as 0.88±0.34 in the control group, 1.35±0.55 in the high-dose group, and 0.65±0.35 in the satellite group. **Conclusion:** This study indicates that MC is safe at therapeutic doses, as no significant toxic effects were observed. Additionally, no delayed effects were noted in the satellite group, suggesting that MC may be suitable for progression to further clinical trials.

**Major Findings:** In the acute toxicity study, no abnormal changes and morbidity were noted, and revealed that the $LD_{50}$ of MC is greater than 2000 mg / Kg b.wt. In the subacute toxicity study, MC did not produce any abnormalities in haematological, biochemical, and histopathological analysis.

**Keywords:** Atomic Absorption Spectroscopy, Heavy Metal, *Maldevi Chenduram*, Organisation for Economic Cooperation and Development, Rat Model, Toxicity Studies

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## 1. Introduction

*Siddha* and other indigenous Indian medical systems have a huge variety of medicinal formulations consisting of herbal, herbo-mineral, and herbo-marine drugs[1]. The herbo-mineral or metallo-mineral preparations have numerous benefits and have widespread usage in the treatment of different chronic ailments by practitioners[2]. In the *Siddha* system, the frequently used dosage forms in combination with metals and minerals are *Parpam*, *Chendhuram*, *Chunnam* and *Pathangam*[3], which are believed to be easily absorbed and assimilated in the body because of their smaller particle size[4]. These comprise good stability, lesser dosage, ease of storability, quick effectiveness and uninterrupted availability[2,5]. Moreover, the purification

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process and the usage of herbal juices in the unique preparation procedures of the medicine tend to reduce the toxic effect of metals and enhance their therapeutic efficacy[6,7].

The dosage form, *Chendhuram,* is an oxidised preparation derived from metals or minerals, characterised by the absence of residual metallic content from its original components[8]. It has high potency and possesses a longer shelf life of 75 years[8]. One among them is *Maldevi Chendhuram* (MC), a metallo-mineral *Siddha* formulation consisting of *Rasam* (Mercury), *Gandhagam* (Sulphur), *Thalagam* (Yellow Orpiment/Arsenic trisulphide), *Lingam* (Red sulphide of mercury), *Ayam* (Iron), *Nagam* (Zinc), *Manosilai* (Red Orpiment) and *Malaiveppilai* (*Melia azedarach* leaf) juice. It is indicated for various ailments like bronchial asthma, pain, venereal diseases, hemiplegia, skin diseases, fever, seizures and nerve weakness in the literature named "*Sigicha Rathna Deepam* Part-2" (SRD) by the author named Kannuswamy Pillai[9]. MC is broadly used by *Siddha* practitioners in their practice for managing *Kabam*-related illnesses, i.e., respiratory disorders like bronchial asthma and nervous disorders. During the COVID-19 pandemic, MC is also extensively employed by *Siddha* practitioners to reduce the severity of viral infection.

The major barrier to the practice of such *Siddha* formulations is the lack of scientific evidence[10]. To gain broader acceptance, there is a need for preclinical and clinical data[11]. A recent literature review highlights that minimal scientific evaluation has been conducted on these preparations. Although preliminary researches have examined the individual ingredients of MC, and studies by Ajantha R *et al.*,[12] have confirmed its acetylcholine inhibitory and anti-inflammatory activities, its overall safety profile remains unestablished. So, the current study was undertaken to assess the acute and sub-acute toxicity profile of MC in Wistar rats as per Organisation for Economic Cooperation and Development (OECD) guidelines.

## 2. Materials and Methods

### 2.1 Drug Profile

The study drug MC was procured from Earth India Naturals, a GMP-certified manufacturing company, and its Batch number is SAB 2001 with a manufacturing date of May 2019.

### 2.2 Quantitative Analysis of Heavy Metals in MC through Atomic Absorption Spectrometry (AAS)

3 g of MC were added to a 300-ml Kjeldahl flask, clamped at 45º. Concentrated nitric acid was used to moisten the substance, followed by the gradual addition of 18 ml of nitric acid with heating. The solution was boiled until it darkened. After cooling, 2 mL of nitric acid was added and heated until darkened. Additional nitric acid was added until no more darkening occurred, then strong heating produced dense, white fumes. The mixture was allowed to cool before adding 5 ml of water and heating it until fumes emerged. Heating was continued until the liquid volume decreased to a few millilitres. Once cooled, another 5 ml of water was added. If the resulting solution appeared yellow, 1 ml of 30% hydrogen peroxide was introduced, and the solution was further evaporated until its volume reached approximately 2–3 ml. If yellow persisted, the process was repeated. The sample was washed with 2% nitric acid, filtered into a 100-ml flask, and diluted to 100 ml with 2% nitric acid, then mixed thoroughly[13].

### 2.3 Animal Selection

The study was conducted at the animal house facility of the National Institute of Siddha, Tambaram Sanatorium, Chennai, following approval from the Institutional Animal Ethics Committee (IAEC No. NIS/IAEC-VIII/17052019/07), and in accordance with OECD guidelines 423 and 407, using Wistar rats. Adult Wistar rats, which are healthy and of both sexes, weighing 140-160g, obtained from CPCSEA approved laboratory, Mass Biotech, Chengalpet, Tamil Nadu, were chosen for this study. The rats were kept in groups inside polypropylene cages. The 12-hour light and 12-hour dark cycles were sustained. The temperature of the room was kept between 22±3°C and the relative humidity between 30–70%. The rats were fed with Rodent pelleted food and RO purified water *ad libitum*. Before the study, the animals were made to acclimatise for 1 week in the laboratory[14].

### 2.4 Dose Determination

According to SRD, the recommended human therapeutic dose of MC ranges from 65 to 130 mg per dose (equivalent to ½ – 1 *Kundri*)[11], administered twice daily along with honey. Therefore, the maximum

recommended human daily dose is 260 mg/day. Assuming an average adult body weight of 70 kg, the human equivalent dose was calculated as 3.71 mg/kg body weight. This dosage was converted to the corresponding rat equivalent using the Paget and Barnes conversion method[15], resulting in 23 mg/kg body weight, which was rounded to 25 mg/kg body weight for the study. Hence, the doses of 25 mg/kg, 125 mg/kg, and 250 mg/kg body weight were chosen for the low, mid, and high dose groups, respectively. The satellite group also received the same dose as high dose group animals (250 mg/kg b.wt.). Adjuvant honey provides synergistic interactions with the active constituents of the drug and may enhance therapeutic efficacy while minimising potential adverse effects.

## 2.5 Acute Toxicity Study

An acute oral toxicity study was conducted as per OECD guidelines 423. At the start, the animals were disseminated into separate cages on the basis of weight and age, then divided into different experimental groups to make the distribution uniform. The female rats were separated into 3 groups with 3 animals in each group, and they were administered with doses of 50, 300, and 2000 mg/kg b.wt of MC, respectively. The animals were fasted for 12 hours, and their body weights were recorded before dosing. A single oral dose of MC was administered using a gavage. Following drug administration, food was withheld for an additional 4 hours. Each female rat was closely observed during the first 30 minutes post-dosing, monitored periodically throughout the first day, with particular attention during the initial 4-hour period and subsequently observed twice daily for a total of 14 days. Mortality, if any, was observed and noted during this period. Bodyweight was noted once a week. Food and water intake were monitored daily. Observations for behavioural signs such as convulsions, tremors, piloerection, sedation, excitation, abnormal gait, motor incoordination, gripping ability, head movements, response to touch, exophthalmos, diarrhoea, salivation, lacrimation, posture, dyspnea, grooming behaviour, and coma were systematically recorded for 14 consecutive days. On the 15$^{th}$ day, all rats were humanely sacrificed under deep anaesthesia and subjected to gross necropsy to examine for any pathological abnormalities[14].

## 2.6 Repeated Dose 28-day Oral Toxicity Study (Sub-acute Oral Toxicity Study)

A total of 50 Wistar rats of both sexes were included in the study. They were divided into 5 groups, with each group consisting of 5 male and 5 female rats. The control group animals were administered honey, and 25, 125, 250, and 250 mg/kg b.wt. of MC were given to the low dose, mid dose, high dose group and satellite group animals, respectively, daily for 28 consecutive days. After drug delivery, all the animals were monitored every day for any death or illness. On the 28$^{th}$ day, all the animals except the satellite group were fasted overnight. The following day, the animals were weighed and euthanised using thiopental sodium (1 mg/100g body weight). Blood was collected from the anaesthetised rats via the jugular vein for haematological and biochemical analysis. Gross pathological changes were examined in all animals. Histopathological examination was carried out on all vital organs and the sciatic nerve in both the control and high-dose treated groups. The satellite group was observed for an additional 14 days without drug administration. At the end of this period, all animals were fasted overnight, weighed, and then sacrificed. From the anaesthetised animals, blood was collected through the jugular vein. Haematological, biochemical and histopathological analyses were done in all satellite group animals[16]. The liver tissues of control, high dose and satellite groups were analysed for heavy metals by AAS.

### *2.6.1 Haematological Analysis*

Complete blood count was investigated in collected blood samples by an automated Bayer Haematology analyser[17].

### *2.6.2. Biochemical Analysis*

Lipid profile, renal function tests and liver function tests were investigated in serum samples using the Mind Ray semi-auto biochemical analyser model BA85A[17].

### *2.6.3 Histopathological Analysis*

The sample was fixed in 10 per cent buffered neutral formalin and trimmed to 3 mm thickness. The tissues were dehydrated, cleared and paraffin-embedded and cut at 3 to 5 µm thickness, stained with hematoxylin and eosin and mounted with DPX for histopathological examinations. Histological slides of organs were made and examined under the microscope[18].

#### 2.6.4 Heavy Metal Analysis of Liver Tissues by Atomic Absorption Spectrometry (AAS)

The concentrations of heavy metals such as mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd) in liver tissues of control, high-dose and satellite group animals were analysed by the Atomic Absorption Spectrometry method[19] using Model AA 240 Series. The test samples were digested using 1 mol/L of HCl for analysis of As and Hg, and with 1 mol/L of $HNO_3$ for the determination of Pb and Cd. The standards for heavy metals were procured from Sigma[20].

#### 2.6.5 Statistical Analysis

The data collected from the study were expressed as mean ± standard deviation. Statistical significance between treatment groups and the control group was evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's multiple range post hoc test. The analysis was performed using GraphPad Instat version 3.0 (GraphPad Software, San Diego, CA), with a p-value of $< 0.05$ considered statistically significant.

## 3. Results

### 3.1 Heavy Metal Analysis of MC using AAS

The heavy metals present in MC were identified and quantified using AAS. The results are presented in Table 1, with measurements expressed in parts per million (ppm).

### 3.2 Acute Toxicity Study

In the acute toxicity study, no death or treatment-related toxicity was observed (Table 2). The animals gained body weight gradually. Additionally, there were no significant differences in food and water consumption among the groups during the study period. Gross necropsy of vital organs revealed no pathological abnormalities across all groups.

**Table 1.** Quantification of heavy metals in MC by AAS

| Sr. No. | Heavy Metals | Results of MC (in ppm) |
|---|---|---|
| 1 | Lead | 0.011 |
| 2 | Mercury | 0.00102 |
| 3 | Arsenic | 0.01002 |
| 4 | Cadmium | 0.00019 |

### 3.3 Repeated Dose 28-day Oral Toxicity Study

In the repeated dose 28-day oral toxicity study, abnormal signs were not observed in behavioural parameters of all the control, test groups and satellite group animals.

#### 3.3.1 Effect of MC on Body Weight, Food and Water Intake

All animal groups showed a consistent, time-dependent increase in body weight, with a statistically significant gain observed in each group compared to the control group. The high-dose group showed a significant increase in food and water intake compared to the control group.

#### 3.3.2 Effect of MC on Haematological Parameters

All haematological parameters remained within normal ranges, and no significant differences were observed between the test groups and the control group. However, some insignificant changes were observed. Inconsistent changes were noted in platelet and MCV. There was a marginal decrease in RBC and Hb in satellite group animals when compared to other groups. MCH was slightly increased in the low dose, while lymphocytes were increased in the mid dose group. WBC showed a small rise in both low-dose and mid-dose groups (Table 3).

#### 3.3.3 Effect of MC on Biochemical Parameters

In the biochemical analysis, a slight increase in total cholesterol was observed in the mid-dose group ($^*p < 0.05$, $p = 0.0007$); however, the value remained within the normal physiological range (Table 4). All other biochemical parameters, including liver function, renal function, and lipid profile, were within normal limits in the test groups compared to the control group.

#### 3.3.4 Histopathological Analysis

Repeated administration of MC did not cause any noticeable pathological alterations in organs such as the brain, heart, lungs, stomach, liver, kidneys, spleen, testes/uterus, ovaries, and sciatic nerve in either the control, high-dose treated, or satellite groups (Figure 1).

### 3.4 Heavy Metal Analysis in Liver Tissues by Atomic Absorption Spectrometry (AAS) Method

Results of Heavy metal content in liver tissues by the AAS method showed that Mercury and Cadmium were

**Table 2.** Effect of *Maldevi Chendhuram* on behavioural parameters of Wistar albino rats

| Sr. No. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Control | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 2 | Test 250 mg/kg /b.wt. | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch response 7. Tremors 8. Decreased Motor Activity 9. Convulsion 10. Muscle spasm 11. Catatonia 12. Muscle relaxant 13.Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality.
(+) Presence of activity; (-) Absence of activity.

**Table 3.** Effect of *Maldevi Chendhuram* on haematological parameters of Wistar albino rats

| Parameters | Control | Low dose | Mid dose | High dose | Satellite |
|---|---|---|---|---|---|
| Red blood cells ($\times10^6$µl) | 7.33±0.99 | 7.41±0.95 | 7.4±0.99 | 7.65±0.94 | 6.85±1.25 |
| White blood cells ($\times10^6$µl) | 8.78±2.90 | 10.01±2.53 | 9.8±2.27 | 8.57±1.75 | 8.6±1.56 |
| Platelet count ($\times10^6$µl) | 642.7 ± 109.80 | 673±53.86 | 628.8±82.31 | 670.5±145.59 | 640±122.25 |
| Haemoglobin (g/dl) | 12.7±1.51 | 13.11±1.58 | 13.03±1.33 | 13.08±1.08 | 11.28±2.70 |
| Mean corpuscular haemoglobin (pg) | 17.50±1.66 | 19.08±2.09 | 17.95±1.9 | 18.11±1.24 | 17.67±2.09 |
| Mean corpuscular volume (fl) | 58.05±6.08 | 66.1±7.18 | 57.93±9.53 | 64.09±10.06 | 60.03±4.95 |
| Neutrophil (%) | 2.89±0.93 | 2.98±0.84 | 2.64±0.73 | 2.45±0.84 | 2.29±0.59 |
| Eosinophils (%) | 1.48±0.22 | 1.66±0.25 | 1.43±0.23 | 1.5±0.34 | 1.5±0.28 |
| Basophils (%) | 0.44±0.42 | 0.26±0.34 | 0.39±0.4 | 0.41±0.4 | 0.35±0.4 |
| Lymphocyte (%) | 69.43±6.75 | 68.86±5.91 | 75.71±10.85 | 73.55±4.95 | 71.99±6.29 |
| Monocytes (%) | 3.27±0.60 | 3.12±0.74 | 2.98±0.83 | 3.25±1.23 | 3.17±0.68 |

Values were expressed as mean± S.D. for N = 10 rats in each group; * - P<0.05; ** - P<0.01.

**Table 4.** Effect of *Maldevi Chendhuram* on biochemical parameters of Wistar albino rats

| Parameters | Control | Low dose | Mid dose | High dose | Satellite |
|---|---|---|---|---|---|
| Total cholesterol (mg/dl) | 112.61±13.25 | 117.07±5.45 | 124.75±9.82* | 108.84±7.43 | 105.95±10.45 |
| High density lipoprotein (mg/dl) | 57.2±6.05 | 55.5±8.3 | 57.4±7.12 | 55.1±3.63 | 50.8±5.33 |
| Low density lipoprotein (mg/dl) | 43.3±8.1 | 45.9±7.68 | 48.5±5.23 | 42±6.86 | 43.7±10.72 |
| Very low-density lipoprotein (mg/dl) | 14.5±2.66 | 15.93±1.53 | 15.03±2.41 | 15.28±2.45 | 13±1.85 |
| Triglycerides (mg/dl) | 31.3±5.58 | 36.5±9.57 | 29.2±8.52 | 37.4±9.92 | 36.8±5.65 |
| Blood urea nitrogen (mg/dl) | 15.4±2.32 | 15±2.58 | 16.1±1.52 | 14.8±1.99 | 13.9±3.70 |
| Creatinine (mg/dl) | 0.69±0.17 | 0.64±0.11 | 0.71±0.12 | 0.62±0.12 | 0.59±0.23 |
| Total Bilirubin (mg/dl) | 0.38±0.11 | 0.36±0.08 | 0.39±0.07 | 0.35±0.11 | 0.33±0.09 |
| Serum glutamic oxaloacetic transaminase (U/L) | 88.2±9.38 | 89.4±12.17 | 79.3±6.25 | 85±10.4 | 83.7±17.49 |
| Serum glutamic pyruvic transaminase (U/L) | 25.3±2.87 | 25±9.53 | 23.4±5.66 | 27.5±5.13 | 28.6±7.14 |

Values were expressed as mean± S.D. for N = 10 rats in each group; * - P<0.05; ** - P<0.01.

found below the detectable level in liver tissues of all the 3 groups, namely control, high-dose and satellite groups. Lead was found only in the high-dose group (1.42±3.47), and it was found below the detectable level in the control and satellite groups. Arsenic content was not significant when the control and high dose groups were compared. But it is significantly reduced in the satellite group in comparison with the high dose group (Table 5).

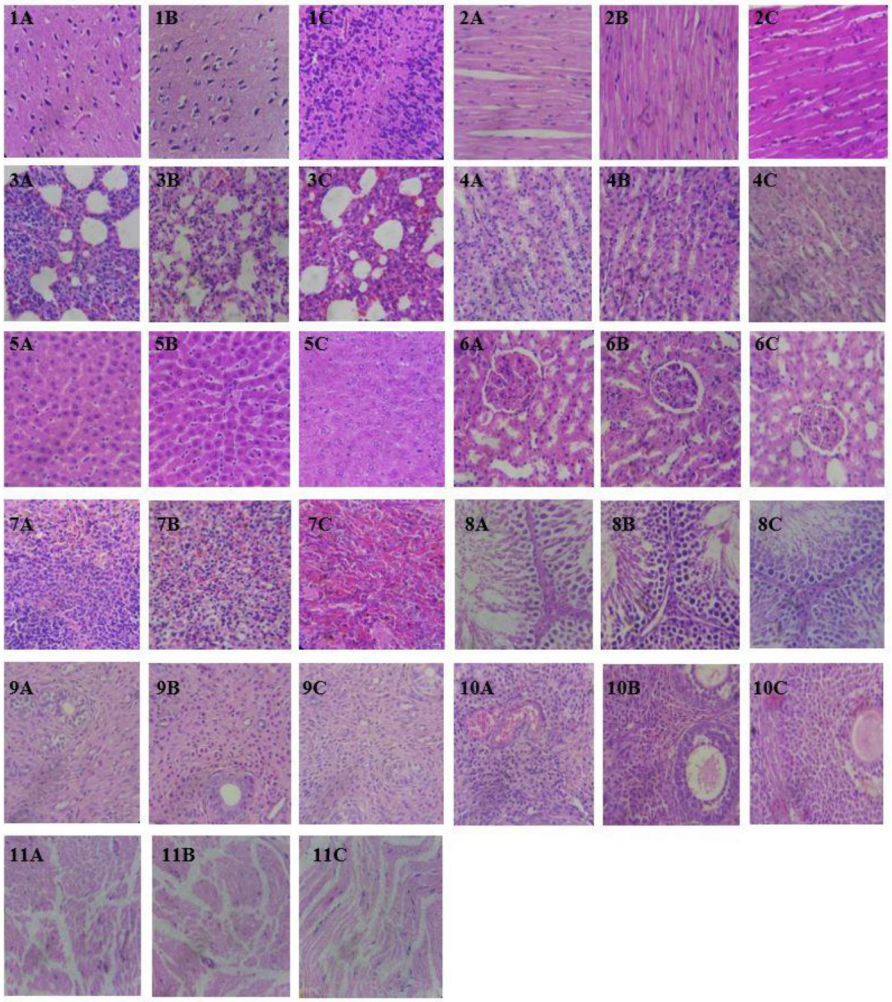



**Figure 1.** Histopathology of control, *Maldevi Chendhuram*-treated rats and satellite group animals in the sub-acute toxicity study. **1A -** Brain (Control); **1B -** Brain (High dose group); **1C -** Brain (Satellite group); **2A** - Heart (Control); **2B** - Heart (High dose group); **2C** - Heart (Satellite group); **3A** - Lung (Control); **3B** - Lung (High dose group); **3C** - Lung (Satellite group); **4A** - Stomach (Control);**4B** - Stomach (High dose group); **4C** - Stomach (Satellite group); **5A -** Liver (Control); **5B** - Liver (High dose group); **5C** - Liver (Satellite group); **6A** - Kidney (Control); **6B** - Kidney (High dose group); **6C** - Kidney (Satellite group); **7A** - Spleen (Control); **7B** - Spleen (High dose group); **7C -** Spleen (Satellite group); **8A** - Testes (Control); **8B** - Testes (High dose group); **8C** -Testes (Satellite group);**9A -** Uterus(Control); **9B -** Uterus (High dose group); **9C** - Uterus (Satellite group); **10A -**Ovary(Control); **10B -** Ovary (High dose group); **10C** - Ovary (Satellite group); **11A -** Sciatic Nerve (Control); **11B -** Sciatic Nerve (High dose group); **11C -** Sciatic Nerve (Satellite group).

**Table 5.** Heavy metal analysis in liver tissues by the Atomic Absorption Spectrometry (AAS) method

| Heavy metal | Control | High Dose | Satellite |
|---|---|---|---|
| Arsenic | 0.88±0.34 | 1.35±0.55 | 0.65±0.35* |
| Lead | BDL | 1.42±3.47 | BDL |
| Mercury | BDL | BDL | BDL |
| Cadmium | BDL | BDL | BDL |

Values are expressed as Mean ± S.D. Statistical significance (p) calculated by one-way ANOVA followed by Tukey-Kramer multiple comparison test (N = 6). *P < 0.05, **P < 0.01 calculated by comparing each pair of groups. BDL - Below detectable limit.

## 4. Discussion

MC is one of the indispensable metallo-mineral formulations used in *Siddha* for treating bronchial asthma, venereal diseases, skin diseases and nervous disorders. Metallo-mineral formulations have long been employed in the *Siddha* system to treat a wide range of serious ailments. The traditional purification and preparation methods are believed to detoxify the metals, making them safe and suitable for human consumption[21]. However, this form of medicine lacks

essential scientific validation on its toxicity profile. So, in this current study, the acute and 28-day sub-acute toxicity study was carried out as per OECD guidelines using Wistar albino rats to assess the probable health hazards likely to occur due to repeated doses and high doses than the therapeutic dose of MC. These findings can also be used to foresee whether MC can cause neurotoxic, nephrotoxic or any vital organ-associated adverse effects. Heavy metal quantification was performed on the test drug, and it was compared with the heavy metal quantification in the liver tissues of experimental rats at the end of the study period. This makes us understand the elimination patterns of the heavy metals present.

An acute toxicity study assesses the adverse effects of a substance following a single exposure or multiple exposures within a short duration[22]. In this study, no abnormal behavioural changes, mortality, or morbidity were noted. No pathological changes were observed in any of the internal organs, indicating that the $LD_{50}$ of MC exceeds 2000 mg/kg body weight. This study shows that even at higher doses that will not be used clinically, MC did not produce any toxic features in the administered rats. So, based on the Globally Harmonised System of Classification and labelling of chemicals, the test drug MC is classified under category 5 or unclassified[14].

In a repeated dose 28-day oral toxicity study, MC did not produce any behavioural changes in all group animals. The food and water intake of all animals gradually increased during the whole of the study period, and no statistically significant changes were seen when compared with the control group. It showed that the drug did not produce any metabolic disturbances in rats. Body weight is an important indicator of the health of an animal. Loss in body weight is the first sign of adverse effects[23]. In this study, there was a gradual time-dependent gain in body weight of all the animals, which is a chief indicator for assessing the overall response of the animal to the administered drug, and the impact of the drug on energy demand, protein synthesis and protein utilisation[24].

Blood is the vital medium of transport for many drugs and xenobiotics in the body and blood cells are highly exposed to toxic compounds[25,26]. So, blood is the sensitive target for toxic compounds and a chief indicator of pathology in both humans and animals[27]. The results of this study showed no significant changes in the haematological parameters of animals treated with the test drug compared to the control group. Similarly, no notable alterations were observed in the satellite group.

Biochemical parameters are major markers in the toxicity study because of their response to toxicants[28]. Results showed no significant changes in biochemical parameters of all test group animals in comparison with the control group, except for a mild increase in total cholesterol of mid-dose group animals. Despite the slight rise in cholesterol level, it comes under the normal physiological range. Hence, it reveals no influential toxic effect of MC.

The kidneys serve as primary detoxification organs, making renal cells highly susceptible to drug-induced damage due to increased exposure[29]. Blood urea nitrogen (BUN) and creatinine levels are considered dependable markers for assessing renal function[30]. In this study, renal function tests revealed no significant alterations in MC-treated animals when compared to the control group. Similarly, liver function was evaluated to determine the potential hepatic effects of MC. Enzymes such as ALT and AST are sensitive indicators of liver damage[31], with elevated levels typically suggesting hepatic dysfunction or disturbances in the biliary system[28]. However, the liver function parameters (ALT, AST, and total bilirubin) remained within normal limits across all test groups. These findings indicate that MC administration did not adversely affect liver or kidney functions, suggesting its safety for therapeutic use.

Histopathological examination plays a crucial role in the toxicological and safety evaluation of drugs, food substances, and chemicals. It enables a detailed and efficient assessment of microscopic changes in the tissues of vital organs, providing valuable insights into potential adverse effects[32]. The study results revealed no abnormal changes in the histopathology of the vital organs in high-dose and satellite group animals in comparison with the control group. Delayed arsenic toxicity can lead to peripheral neurotoxicity, which may result from both acute and chronic exposure[33]. Yilmaz S *et al.,* found that histopathologically, arsenic compounds cause degeneration of the sciatic nerves[34]. In this study, the analysis of sciatic nerves from control, high-dose, and satellite groups of animals showed no

histopathological abnormalities, indicating that MC has no adverse impact on peripheral nerves, even after 28 days of exposure.

Further, the safety of the drug was confirmed by comparing the heavy metal content in the test drug with the heavy metal content in liver tissues of control, high dose and satellite groups by the AAS method. As shown in Table 1, the heavy metal analysis of MC through AAS showed the presence of Lead (0.011 ppm), Mercury (0.00102 ppm), Arsenic (0.01002 ppm) and Cadmium (0.00019 ppm). The results of liver tissue analysis showed that the mercury and cadmium contents were found below the detectable level in the liver tissues of the above-mentioned group of animals. Lead was found only in the high dose group animal (1.42±3.47). But it was found below the detectable level in liver tissues of the control and satellite groups, which showed that the accumulation of lead in liver tissues was eliminated in the satellite group during the post-retrieval period. Arsenic was found at the level of 0.88±0.34, 1.35±0.55 and 0.65±0.35 in the control, high dose and satellite groups, respectively. Arsenic content found in the liver tissues of the control group may be due to the water or food source. Arsenic level did not diverge significantly between control and high dose groups. But the arsenic content was statistically decreased in the liver tissues of the satellite group when compared with the high dose group. The result showed that MC did not cause damage to the tissues due to the elimination of the drug during the post-retrieval period. The safety of MC is hence established.

## 5. Conclusion

In accordance with the study results, it is evident that the NOAEL (No Observed Adverse Effect Level) of MC was found to be 250 mg/Kg b.wt. This dose level is ten times higher than the therapeutic dose, which is not likely to be used in clinical practice. So, the therapeutic dose of MC, 65-130 mg (1/2-1 Kundri), along with the adjuvant honey, which is stated in the *Siddha* literature, is safe to be consumed by humans. Further chronic toxicity, mutagenicity and genotoxicity studies of the test drug are needed to prove its safety. At the next level, pharmacological studies and clinical trials can be carried out for the establishment of its clinical significance.

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## Author Contribution

B.N. – Experimental research work, results and observations, statistical analysis, and manuscript drafting; G.J. – Experimental research work; T.S. and I.S. – Manuscript drafting and manuscript review; P.S. and R.M. – Final approval of research work and final revision of manuscript. All authors have read and agreed to the published version of the manuscript.