Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/mzcgdx67

PRECLINICAL SAFETY EVALUATION OF *MANDOORATHY MATHIRAI*: *IN VITRO* CYTOCOMPATIBILITY AND *IN VIVO* ACUTE AND SUB-ACUTE TOXICITY ASSESSMENT IN RODENT MODELS

Manikandan B[1*], Elansekaran S[2], Uma K.S[3]

[1*]Associate professor, Maria Siddha Medical College and Hospital, Attur, Kanniyakumari District 629177, Tamil Nadu, India.
[2]Professor, Department of Siddha, National institute of Siddha, Chennai, Tamil Nadu, India.
[3]Associate professor, Department of Siddha, The Tamil Nadu Dr.MGR Medical University, Chennai, Tamil Nadu, India.

***Corresponding Author:** Dr. B. Manikandan
*Lecturer, Maria Siddha Medical College and Hospital, Attur, Kanniyakumari District 629177, Tamil Nadu, India.

Abstract

Background: *Mandoorathy Mathirai* (MM) is a classical Siddha formulation used traditionally for managing hepatic and haematological disorders. Despite its therapeutic potential, its safety profile has not been thoroughly established through systematic preclinical evaluation.

Objective: To assess the in vitro cytocompatibility, and in vivo acute and sub-acute toxicity profile of MM in rodent models, with a focus on haematological, hepatic, renal, lipid, and organ weight parameters.

Materials and Methods: Cytotoxicity of aqueous (CAE), methanolic (CME), and chloroform (CCE) extracts of MM was evaluated in murine splenocytes, hepatocytes, and thymocytes using the MTT assay (20–100 µg/mL). Acute oral toxicity was assessed in Wistar rats according to OECD 423 guidelines. Sub-acute oral toxicity was evaluated following OECD 407, with rats receiving 12, 59, or 117 mg/kg MM daily for 28 days. Body weight, food and water intake, haematology, serum biochemistry (hepatic, renal, lipid profiles), and organ weights were measured.

Results: MTT assay results showed high cell viability (>87%) in all extracts across all concentrations, indicating negligible cytotoxicity. Acute toxicity studies revealed no mortality or clinical abnormalities at 2000 mg/kg. In sub-acute studies, MM produced no adverse effects on body weight, feed/water intake, haematological indices, hepatic and renal biomarkers, lipid profile, or relative organ weights. Minor variations observed (e.g., slight HDL increase, marginal chloride elevation) remained within physiological limits and had no associated pathological findings.

Conclusion: MM demonstrated an excellent in vitro cytocompatibility profile and in vivo safety in both acute and sub-acute studies, supporting its safe use at therapeutic doses and justifying further pharmacological and clinical evaluations.

Keywords: *Mandoorathy Mathirai*, Siddha medicine, toxicity, safety evaluation, OECD guidelines, cytocompatibility.

1.Introduction

Herbo-mineral formulations occupy a central role in the Siddha system of medicine, a traditional healthcare system practised primarily in South India. These formulations combine purified mineral or metallic components with medicinal plants to produce synergistic therapeutic effects. Among them, *Mandoorathy Mathirai* (MM) is a well-known preparation traditionally prescribed for the management of hepatic ailments, anaemia, and conditions associated with general weakness and debility[1,2]. MM contains purified *Mandoor* (iron oxide) as its core mineral ingredient, combined with selected herbs that are reputed to enhance bioavailability and therapeutic efficacy. According to Siddha literature, MM is believed to correct deranged *Uyir Thathu* (vital principles), restore haemopoietic balance, and improve organ function, particularly of the liver and spleen [3]. The iron content in *Mandoor*, once properly processed through traditional detoxification techniques (*suddhi*), is thought to contribute to haemoglobin synthesis, improve oxygen transport, and support overall vitality.

The therapeutic reputation of MM is largely based on centuries of empirical use and its inclusion in classical Siddha pharmacopoeias. However, reliance solely on traditional claims without scientific validation is increasingly challenged in the context of evidence-based medicine. Modern healthcare frameworks require rigorous safety and efficacy data, particularly for herbo-mineral formulations, due to concerns over heavy metal toxicity, batch-to-batch variation, and the impact of improper preparation methods [4]. Indeed, while many herbal and herbo-mineral medicines are perceived as safe, adverse outcomes have been reported when raw materials are inadequately purified, contaminated with environmental toxins, or dosed inappropriately [5]. Such risks underscore the necessity of formal toxicological evaluation before widespread clinical adoption.

Internationally, the safety of traditional medicines is increasingly governed by regulatory requirements that align with guidelines set forth by the Organisation for Economic Co-operation and Development (OECD) and the World Health Organization (WHO). These frameworks emphasise a stepwise evaluation process, beginning with in vitro cytotoxicity screening to assess potential cellular toxicity, followed by in vivo acute and sub-chronic toxicity studies in relevant animal models [6,7]. Such studies allow researchers to identify target organs of toxicity, establish no-observed-adverse-effect levels (NOAELs), and inform safe dose ranges for clinical use.

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay remains one of the most widely used in vitro methods for determining cell viability and cytocompatibility. The assay is based on the ability of metabolically active cells to reduce MTT to insoluble formazan crystals via mitochondrial dehydrogenases, providing a quantitative measure of viable cell populations [8]. For herbo-mineral formulations like MM, MTT assays in primary cell cultures such as splenocytes, hepatocytes, and thymocytes can help assess potential toxicity to immune and hepatic cell types, which are critical to both therapeutic efficacy and systemic safety.

Beyond in vitro testing, in vivo safety assessments provide a holistic view of a formulation's physiological impact. Acute oral toxicity studies, typically conducted in rodents, identify immediate toxic effects and establish approximate lethal doses. Sub-chronic toxicity studies, spanning 28–90 days, evaluate the effects of repeated dosing on multiple organ systems. According to OECD guidelines, parameters monitored include body weight progression, food and water intake, clinical observations, and mortality, along with laboratory investigations of haematology, serum biochemistry, and urinalysis [9]. Post-mortem evaluations involve gross pathological examinations, organ weight measurements, and histopathology to detect any microscopic tissue alterations [10]. This multi-tiered approach ensures a comprehensive evaluation of systemic safety. While MM is traditionally regarded as safe when prepared according to classical Siddha protocols, systematic safety data are scarce. Given the formulation's mineral component, it is particularly important to confirm the absence of adverse effects on hepatic, renal, and haematological functions, as these systems are often most susceptible to heavy metal exposure. Additionally, the lipid profile and organ weight assessments provide further insight into possible subclinical metabolic disturbances or organ-specific effects. In this context, the present study was designed to evaluate both the in vitro cytocompatibility and the in vivo acute and

sub-acute toxicity profile of MM. The cytocompatibility study employed MTT assays on murine splenocytes, hepatocytes, and thymocytes to determine effects on key immune and metabolic cell types. The in vivo component followed OECD guidelines to assess the impact of repeated oral administration of MM in Wistar rats, monitoring body weight, food and water consumption, haematological parameters, hepatic and renal function indices, lipid profile, and relative organ weights. This integrative approach aims to generate a robust preclinical safety dataset, thereby validating the formulation's safety for continued clinical use and informing future pharmacological and clinical research.

2.Materials and Methods

2.1.*In Vitro* Cytotoxicity (MTT Assay)

Primary splenocytes, hepatocytes, and thymocytes were isolated from healthy BALB/c mice (6–8 weeks old, 20–25 g) under aseptic conditions, with spleen, liver, and thymus immediately excised and transferred to chilled phosphate-buffered saline (PBS, pH 7.4) containing 1% antibiotic–antimycotic solution. Splenocytes and thymocytes were obtained by gently teasing the organs through a 70 µm sterile nylon mesh to yield single-cell suspensions, which were centrifuged at 1500 rpm for 10 min at 4 °C, and the pellets resuspended in complete RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Hepatocytes were prepared using a two-step collagenase perfusion method, wherein the liver was perfused with calcium-free Hank's Balanced Salt Solution (HBSS) followed by HBSS containing 0.05% collagenase type IV at 37 °C, filtered through a nylon mesh, and centrifuged at 50 × g for 5 min to pellet viable cells. Cell viability was assessed by trypan blue exclusion, and only suspensions with >95% viability were used [11,12]. Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated for 24 h at 37 °C with 5% CO_2. Aqueous (CAE), methanolic (CME), and chloroform (CCE) extracts of *Mandoorathy Mathirai* were prepared by maceration, concentrated under reduced pressure, and dissolved in dimethyl sulfoxide (DMSO) ensuring a final well concentration ≤ 0.5% v/v. Cells were treated with 20, 40, 60, 80, or 100 µg/mL of each extract in triplicate for 24 h, with untreated wells serving as controls. Cytotoxicity was assessed by Mosmann's MTT method [13,14], wherein 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and incubated for 4 h, after which the medium was aspirated and the formazan crystals dissolved in 100 µL of DMSO. Absorbance was recorded at 570 nm with background correction at 630 nm using a microplate reader, and cell viability (%) was calculated as $(OD_{teated}/ODc_{ontrol}) \times 100$, with results expressed as mean ± SD from triplicate experiments.

2.2.Acute and Sub-acute Toxicity

Wistar rats (n = 10 per group, equal numbers of males and females) were housed under standard laboratory conditions (22 ± 2 °C, 55 ± 5% relative humidity, 12 h light/dark cycle) with ad libitum access to standard pellet diet and water, and acclimatised for 7 days prior to experimentation. Acute oral toxicity was assessed following OECD guideline 423 [6], where a single oral dose of *Mandoorathy Mathirai* (MM) at 2000 mg/kg body weight was administered via gavage to the treatment group, while controls received vehicle alone; animals were observed individually during the first 30 min, periodically for the next 4 h, and then daily for 14 days for mortality, behavioural alterations, autonomic and central nervous system effects, and general health. For sub-acute toxicity, OECD guideline 407 [7] was followed, with separate groups receiving MM at doses of 12, 59, or 117 mg/kg body weight once daily by oral gavage for 28 consecutive days, and controls receiving vehicle only; all animals were observed daily for clinical signs of toxicity and behavioural changes, and body weight, food, and water intake were recorded weekly. At study termination, animals were fasted overnight, anaesthetised, and blood was collected via retro-orbital plexus for haematological analysis (red blood cell count, haemoglobin concentration, white blood cell count, platelet count, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, erythrocyte sedimentation rate, and differential leucocyte count) using an automated

haematology analyser, and for serum biochemical parameters (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, total and direct bilirubin, total protein, albumin, globulin, urea, creatinine, uric acid, total cholesterol, triglycerides, high-density and low-density lipoproteins, very-low-density lipoproteins, and electrolytes including sodium, potassium, and chloride) using standard enzymatic kits [15,16]. Following blood collection, animals were euthanised, and major organs including liver, kidney, heart, lungs, spleen, brain, stomach, and gonads were excised, blotted dry, weighed to determine relative organ weight, and examined macroscopically for gross pathological changes; representative samples were fixed in 10% neutral buffered formalin for histopathological evaluation after routine processing, paraffin embedding, sectioning at 5 µm thickness, and staining with haematoxylin and eosin for microscopic examination under a light microscope.

2.5.Histopathology

For histopathological examination, representative tissue samples of liver, kidney, spleen, and heart from all experimental groups were carefully excised immediately after sacrifice, rinsed gently in ice-cold physiological saline to remove residual blood, and fixed in 10% neutral buffered formalin for a minimum of 48 hours to preserve structural integrity [17,18]. Fixed tissues were processed through graded ethanol series for dehydration (70%, 80%, 90%, 95%, and 100%), cleared in two changes of xylene, and embedded in molten paraffin wax at 58–60 °C using a paraffin embedding station. Paraffin blocks were sectioned at 5 µm thickness using a rotary microtome, and ribbons of sections were floated on a warm water bath (45 °C) to remove wrinkles before being mounted on clean glass slides pre-coated with Mayer's albumin. The sections were dried overnight at 37 °C, followed by deparaffinisation in xylene and rehydration through descending grades of ethanol (100%, 95%, 80%, 70%) to distilled water. Staining was performed using Harris's haematoxylin for nuclear detail, followed by differentiation in acid alcohol, bluing in alkaline water, and counterstaining with eosin for cytoplasmic and extracellular matrix components. Stained sections were dehydrated through ascending grades of ethanol, cleared in xylene, and mounted with DPX mounting medium under coverslips. Microscopic examination was carried out using a light microscope at magnifications ranging from ×100 to ×400 by a veterinary pathologist blinded to treatment groups, assessing parameters such as hepatocyte morphology, sinusoidal architecture, presence or absence of necrosis, fatty change, inflammatory infiltrates, vascular congestion, and restoration of normal tissue architecture. Representative photomicrographs were captured for documentation and comparative analysis between treated and control groups.

3.Results

3.1. Effect of *Mandoorathy Mathirai* on Cell viability of Splenocytes

MTT assay results on murine splenocytes revealed that all three extracts of *Mandoorathy Mathirai*—aqueous (CAE), methanolic (CME), and chloroform (CCE)—maintained high cell viability (>90%) across the tested concentrations (20–100 µg/mL), with only a slight, concentration-dependent reduction from control values. At the highest dose tested (100 µg/mL), CAE, CME, and CCE showed viabilities of 90.45%, 91.48%, and 91.15%, respectively, indicating minimal cytotoxicity and negligible differences between extraction solvents. These findings confirm that MM is non-toxic to splenocytes within the evaluated concentration range, supporting its cytocompatibility for further in vitro and in vivo pharmacological investigations as shown in table 1.

Table 1:The cytotoxic effect of *Mandoorathy Mathirai* on Splenocytes

Concentration (µg/ml)	Cell viability (%)		
	Combined Aqueous extract	Combined Methanolic extract	Combined Chloroform extract
Control	100	100	100
20	98.56	97.82	98.34
40	95.21	96.16	96.15
60	94.64	95.48	95.25
80	92.16	93.79	93.55
100	90.45	91.48	91.15

3.2. Effect of *Mandoorathy Mathirai* on Cell viability of hepatocytes

MTT assay evaluation on murine hepatocytes showed that aqueous (CAE), methanolic (CME), and chloroform (CCE) extracts of *Mandoorathy Mathirai* maintained high cell viability (>88%) across concentrations from 20–100 µg/mL, with a gradual, dose-dependent reduction compared to the control. At 20 µg/mL, CAE, CME, and CCE exhibited viabilities of 95.55%, 96.45%, and 95.75%, respectively, while at the maximum concentration (100 µg/mL) the values were 89.37%, 88.87%, and 88.48%. The small variation among extracts suggests that the choice of solvent had minimal impact on hepatocyte compatibility. These findings indicate that MM is non-toxic to primary hepatocytes within the tested concentration range, supporting its hepatic cell safety profile for further pharmacological investigations as shown in table 2.

Table 2: The cytotoxic effect of *Mandoorathy Mathirai* on Hepatocytes

Concentration (µg/ml)	Cell viability (%)		
	Combined Aqueous extract	Combined Methanolic extract	Combined Chloroform extract
Control	100	100	100
20	95.55	96.45	95.75
40	93.15	94.76	93.48
60	91.56	92.68	91.48
80	90.48	90.18	89.86
100	89.37	88.87	88.48

3.3. Effect of *Mandoorathy Mathirai* on Cell viability of thymocytes

MTT assay results on murine thymocytes demonstrated that aqueous (CAE), methanolic (CME), and chloroform (CCE) extracts of *Mandoorathy Mathirai* retained high cell viability (>87%) across the tested concentration range (20–100 µg/mL), with a mild, concentration-dependent decline from control values. At 20 µg/mL, CAE, CME, and CCE showed viabilities of 96.55%, 95.85%, and 94.65%, respectively, while at the highest dose (100 µg/mL), viabilities were 88.67%, 87.96%, and 87.48%. The close similarity in results across extracts suggests minimal solvent influence on thymocyte compatibility. Overall, these findings indicate that MM is non-toxic to primary thymocytes within the tested range, confirming its cytocompatibility for immune cell applications in further studies as shown in table 3.

Table 3: The cytotoxic effect of *Mandoorathy Mathirai* on Thymocytes

Concentration (µg/ml)	Cell viability (%)		
	Combined Aqueous extract	Combined Methanolic extract	Combined Chloroform extract
Control	100	100	100
20	96.55	95.85	94.65
40	94.25	92.68	92.68
60	91.45	90.46	90.96
80	89.35	88.32	88.65
100	88.67	87.96	87.48

3.4. Effect of *Mandoorathy Mathirai* on Acute and Sub-Acute Toxicity study

Sub-acute toxicity evaluation of *Mandoorathy Mathirai* in Wistar rats showed that administration at doses of 12, 59, and 117 mg/kg body weight for 28 days produced no significant adverse effects on body weight, food intake, or water intake compared to the control group. Mean body weights across groups remained consistent, ranging from 131.16 ± 8.23 g to 134.27 ± 9.14 g, with no dose-dependent decrease. Daily food intake values varied slightly between 37.12 ± 2.84 g and 43.56 ± 1.85 g, and water intake ranged from 43.05 ± 2.75 ml to 51.35 ± 2.98 ml, with fluctuations within physiological limits. These findings indicate that repeated oral administration of MM did not impair growth performance or alter feeding and drinking behaviour, supporting its safety in sub-acute exposure conditions.

Table 4: Effect of *Mandoorathy Mathirai* on Body weight, Food and Water intake of treated rats

Dose (mg/kg)	Body weight (g)	Food intake (g/day)	Water intake (ml/day)
Control	132.22±1.89*	37.18±1.96	43.05±2.75
12	133.26 ±4.96*	37.12±2.84	51.35±2.98
59	134.27±9.14	41.46±1.25	47.07±3.27
117	131.16±8.23*	43.56±1.85	50.24±2.44

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

3.5. Haematological, Hepatic, and Renal Safety Profile of *Mandoorathy Mathirai* in Wistar Rats

Mandoorathy Mathirai (MM) at doses of 12, 59, and 117 mg/kg for 28 days produced no adverse alterations in haematological, hepatic, or renal parameters compared to controls, indicating an overall favourable safety profile. Haematological analysis showed that red blood cell count, haemoglobin concentration, leukocyte count, platelet count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) remained within normal limits across all treated groups, with only minimal variation from control values. Differential leucocyte count (DLC) parameters, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were stable, with a minor increase in eosinophil percentage at higher doses, likely reflecting physiological variation rather than treatment-related toxicity.

Hepatic biochemical indices, including total and direct bilirubin, indirect bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total protein, albumin, globulin, albumin/globulin ratio, and gamma-glutamyl transferase (GGT), showed no significant deviations from control levels, indicating preserved hepatic function. Similarly, renal function tests revealed stable urea, creatinine, uric acid, and electrolyte (sodium, potassium, chloride) levels, with only a marginal increase in chloride concentration at the highest dose that remained within the physiological range. Overall, the consistency of haematological, hepatic, and renal markers across treatment groups demonstrates that repeated oral administration of MM does not adversely affect blood cell indices, liver enzymatic activity, protein metabolism, or kidney function, supporting its safety under sub-acute exposure conditions.

Table 5: Effect of *Mandoorathy Mathirai* on Haematological parameters

Parameter	Control	12 mg/kg	59 mg/kg	117 mg/kg
Red blood cell (mm^3)	7.35±0.14	7.23±0.31	7.50±0.16	7.61±0.13
HB (%)	14.26±0.24	14.82±0.23	15.45±0.27	15.89±0.21
Leukocyte ($x10^6$/mL)	10245±115.51	10252±121.05	10301±125.35	10320±2132.14
Platelets/ul	1285±14.32	1242±25.34	1245±15.27	1246±17.23
MCV (gl)	57.32±2.21	56.16±1.56	56.42±1.17	55.04±1.28
DLC (%)				
N	4.52±1.32	5.32±3.05	4.85±1.14	5.26±1.01

L	90.12±4.06	90.42±3.17	90.12±3.52	90.14±2.68
M	2.11±1.23	2.27±1.45	2.24±0.84	2.32±0.64
E	1.12±0.32	1.24±0.30	1.43±0.28	1.48±0.17
B	0	0	0	0
ESR(mm)	1±00	1±00	1±00	1±00
PCV	48.20±2.38	47.32±3.21	47.14±2.95	47.30±2.74
MCH pg	18.58±1.52	17.84±0.88	18.85±00.65	18.91±0.21
MCHC g/dl	31.67±1.23	32.04±0.85	32.18±2.63	31.69±1.35

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

Table 6: Effect of *Mandoorathy Mathirai* on Hepatic parameters

Dose (mg/kg)	Control	12 mg/kg	59 mg/kg	117 mg/kg
Total Bilirubin (mg/dL)	0.204±1.01	0.210±0.12	0.205±0.14	0.201±1.02
Bilirubin direct (mg/dL)	0.1±0.1 7	0.15±0.1 5	0.1±0.1 9	0.12±0.14
Bilirubin indirect(mg/dL)	0.1±00	0.12±00	0.1±00	0.17±00
SGOT (U/L)	165.44±3.14	164.88±2.52	161.72±2.56	164.34±2.12
SGPT(U/L)	45.42±2.05	44.4±2.28	43.47±2.15	44.65±4.13
Total Protein(g/dl)	10.62±1.30	10.16±0.30	10.35±0.27	10.11±0.46
Albumin(g/dl)	3.28±0.18	3.26±0.22	3.35±0.20	3.24±0.15
Globulin(g/dl)	5.98±0.24	5.24±0.30	5.77±0.34	5.48±0.10
A/G Ratio(g/dl)	0.55±0.21	0.54±0.21	0.58±0.41	0.63±0.5 0
GGT(U/L)	7.4±0	7.3±0.3	7.6±0.2	7.32±0

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

Table 7: Effect of *Mandoorathy Mathirai* on Renal parameters

Dose (mg/kg)	Control	12 mg/kg	59 mg/kg	117 mg/kg
Urea(mg/dL)	63.24±3.09	61.36±5.01	60.22±1.25	62.62±1.27
Creatinine (mg/dL)	0.85±0.18	0.86±0.21	0.92±0.23	0.82±0.26
Uric acid (mg/dL)	1.7±0.17	1.8±0.21	1.7±0.24	1.64±0.22
Na (m.mol)	137.21±3.04	136.4±3.32	137.45±3.12	138.14±1.98
K (m.mol)	21.50±2.43	20.51±2.17	21.10±2.25	21.23±2.18
Cl (m.mol)	98.54±2.05	99.10±4.17	97.28±3.57	99.20±5.35*

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

3.6. Lipid Profile and Organ Weight Assessment Following 28-Day Oral Administration of *Mandoorathy Mathirai* in Wistar Rats

Lipid profile assessment showed that total cholesterol, HDL, LDL, VLDL, triglycerides, TC/HDL ratio, and blood glucose levels remained within normal physiological limits across all tested doses (12, 59, and 117 mg/kg). Slight numerical variations were observed, such as a modest rise in total cholesterol at the highest dose (45.83 ± 3.12 mg/dL) and a corresponding small increase in HDL (13.75 ± 3.00 mg/dL), but these were not of toxicological concern. LDL levels showed a mild decline at 117 mg/kg, while triglyceride and VLDL values remained stable across groups, and fasting blood glucose levels did not deviate significantly from controls, suggesting that MM does not disturb carbohydrate or lipid metabolism under the tested conditions.

Organ weight evaluation revealed that the absolute weights of the liver, heart, lung, spleen, ovary, testes, brain, kidney, and stomach in treated groups were comparable to those of the control group, with no statistically significant pathological enlargement or atrophy. Minor variations, such as a slightly higher liver weight at 117 mg/kg and marginal fluctuations in kidney weight, were within normal biological variation and not associated with any gross pathological changes. These findings confirm that repeated oral administration of MM at the tested doses does not induce dyslipidaemia or organ hypertrophy/atrophy, supporting its overall sub-acute safety profile.

Table 8: Effect of Mandoora Chooranam on Lipid profile

Dose (mg/kg)	Control	12 mg/kg	59 mg/kg	117 mg/kg
Total cholesterol (mg/dL)	41.38±1.35	40.72±1.02	43.83±2.12	45.83±3.12
HDL (mg/dL)	12.93±1.45	12.28±1.36	12.95±1.20	13.75±3.00
LDL(mg/dL)	38.65±2.88	42.96±3.18	37.36±2.47	35.04±1.88
VLDL(mg/dl)	16.88±2.46	16.64±2.10	16.62±1.66	14.92±1.13
Triglycerides (mg/dl)	82.96±1.23	81.74±1.35	81.72±1.110	83.84±1.36
TC/HDL ratio (g/dl)	3.63±2.21	3.53±1.26	3.96±2.27	3.74±4.28
Blood glucose(mg/dl)	110.85±8.62	112.75±3.33	112.37±4.12	112.42±2.58

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

Table 9: Effect of *Mandoorathy mathirai* on organ weight

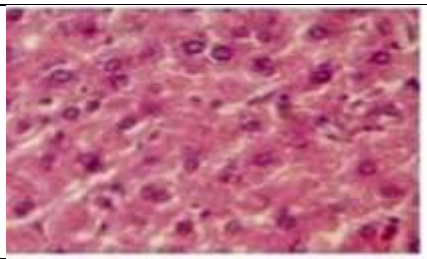
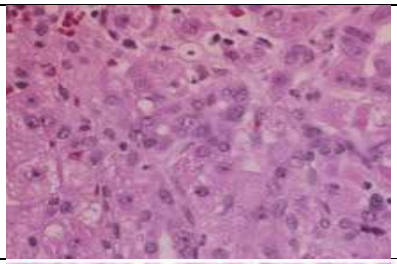
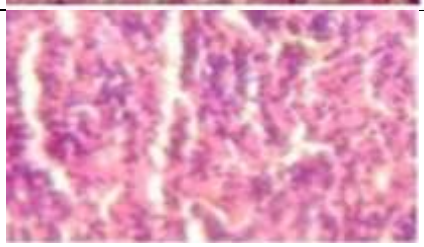
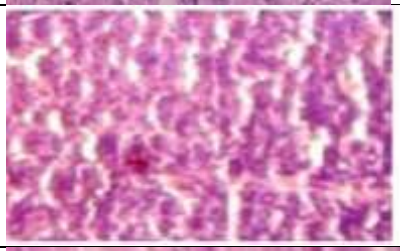
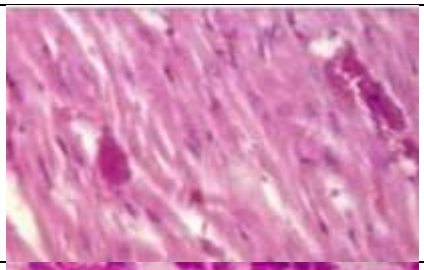
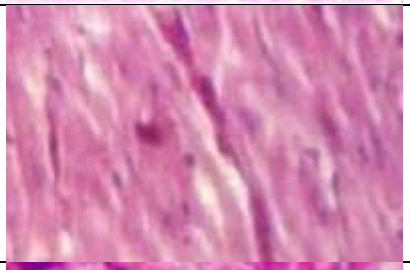
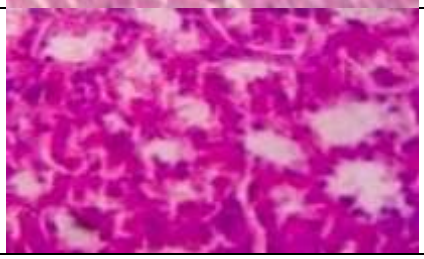
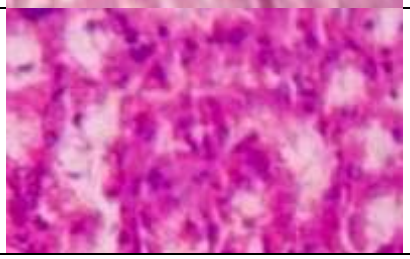
Dose (mg/kg)	Control	12 mg/kg	59 mg/kg	117 mg/kg
Liver (g)	5.86±0.14	5.82±0.21	6.45±0.20	7.21±0.22
Heart (g)	0.84±0.05	0.80±0.02	0.78±0.05	0.85±0.02
Lung (g)	1.79±0.25	1.74±0.21	1.72±0.22	1.79±0.10
Spleen (g)	0.75±0.07	0.68±0.05	0.69±0.05	0.66±0.04
Ovary (g)	1.91±0.14	1.86±0.12	1.67±0.18	1.66±0.15
Testes (g)	1.40±0.12	1.41±0.12	1.42±0.12	1.43±0.19
Brain (g)	1.43±0.18	1.49±0.17	1.44±0.16	1.44±0.18
Kidney (g)	0.70±0.05	0.82±0.04	0.72±0.05	0.72±0.04
Stomach (g)	1.23±0.10	1.12±0.20	1.30±0.17	1.23±0.12

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01 *vs control* N=10.

3.7. Effect of *Mandoora Chooranam* on organ Histology

Histological examination of vital organs, including the liver, spleen, heart, and kidneys, from control and high-dose *Mandoorathy Mathirai* (MM)-treated rats revealed no evidence of treatment-related abnormalities. In the liver (Panel 1), both control (Figure A) and high-dose treated (Figure B) groups exhibited preserved hepatic architecture, with intact hepatocyte morphology and sinusoidal arrangement. Spleen sections (Panel 2) from treated animals showed normal trabeculae and capsule integrity comparable to controls. Cardiac tissue (Panel 3) displayed well-preserved myocardial fibres and nuclei of myocytes in both control and treated groups, with no signs of degeneration or inflammatory infiltration. Renal sections (Panel 4) demonstrated intact glomeruli, Bowman's capsule, and capillary networks, with no pathological alterations in the treated group relative to controls. Overall, macroscopic examination revealed no gross pathological lesions, and microscopic analysis confirmed that high-dose MM administration did not induce histoarchitectural changes in the examined organs, supporting its non-toxic profile under the study conditions.

Figure 1: Histopathological evaluation of vital organs in control and high-dose *Mandoorathy Mathirai* (MM)-treated Wistar rats.

S. No.	Organs	MM Normal Control	MM_HIGH DOSE
1	Liver		
2	Spleen		
3	Heart		
4	Kidney		

Panel 1: Liver – *(A)* Control showing normal hepatic architecture; *(B)* High-dose treated group exhibiting intact hepatocytes and preserved sinusoidal arrangement without pathological changes. Panel 2: Spleen – *(A)* Control with normal trabeculae and capsule; *(B)* High-dose treated group with no structural alterations in trabeculae or capsule integrity. Panel 3: Heart – *(A)* Control showing well-organised myocardium; *(B)* High-dose treated group with preserved myocyte nuclei and myocardial fibres, free from degeneration or inflammation. Panel 4: Kidney – *(A)* Control with normal glomeruli, Bowman's capsule, and capillary structure; *(B)* High-dose treated group displaying intact renal architecture without detectable abnormalities.

4.Discussion

The present study provides an integrated preclinical safety evaluation of *Mandoorathy Mathirai* (MM), a classical Siddha herbo-mineral formulation traditionally used for hepatic disorders, anaemia, and general debility. Using a combination of in vitro cytocompatibility assays and in vivo acute and sub-acute toxicity models, we demonstrate that MM exhibits a favourable safety profile, thereby supporting its continued clinical relevance when prepared according to standard Siddha purification protocols. Our in vitro MTT assays revealed high cell viability in primary splenocytes, hepatocytes, and thymocytes, with minimal decline (<13%) even at the highest tested concentration (100 µg/mL) across aqueous, methanolic, and chloroform extracts. These results indicate a low

cytotoxic potential towards immune and hepatic cell populations, aligning with previous reports that traditionally purified *Mandoor* preparations exhibit reduced free radical generation and negligible mitochondrial impairment in mammalian cells [19]. The lack of significant solvent-dependent variation further suggests that the observed cytocompatibility is intrinsic to the formulation rather than an artefact of extraction chemistry.

The acute oral toxicity study (OECD 423) confirmed the absence of mortality or behavioural abnormalities at a single dose of 2000 mg/kg, classifying MM as "unclassified" in terms of acute toxicity under the Globally Harmonized System (GHS). Sub-acute administration (OECD 407) at doses up to 117 mg/kg for 28 days produced no adverse effects on body weight progression, food and water intake, or clinical condition. Haematological indices remained within physiological limits, with stable erythrocytic, leukocytic, and platelet parameters, suggesting no myelotoxic or immunosuppressive effects. The slight, non-significant increase in eosinophil percentage at higher doses likely reflects normal biological variability rather than allergenic or inflammatory responses, as eosinophilia in rodent models is often transient and non-pathological [20].

Liver function tests (SGOT, SGPT, bilirubin profile, total protein, albumin/globulin ratio) remained stable, consistent with preserved hepatocellular integrity and absence of cholestatic injury. This is particularly relevant for *Mandoor*-based preparations, as poorly processed iron oxides can trigger oxidative hepatotoxicity [21]. Similarly, renal parameters (urea, creatinine, uric acid, electrolytes) remained unaltered, indicating no nephrotoxic liability. The lipid profile was not adversely affected, with cholesterol, triglycerides, and glucose values remaining within normal ranges, supporting the absence of metabolic disturbances.

Histopathological analysis further corroborated the biochemical findings. Liver sections from MM-treated animals retained normal lobular architecture with intact hepatocyte morphology, while kidneys showed preserved glomerular and tubular structures. Spleen and heart tissues exhibited normal histoarchitecture, without inflammatory infiltration or degenerative changes. Such congruence between clinical chemistry and histology strengthens the evidence that MM does not elicit subclinical organ damage under the tested conditions [22,23].

Our findings resonate with earlier work on other Siddha herbo-mineral formulations, where proper purification (*suddhi*) and controlled dosing were shown to mitigate heavy metal toxicity risks while preserving therapeutic efficacy [24]. The absence of organ weight changes and the stable haematological, biochemical, and histological profiles observed here are consistent with the hypothesis that the mineral content in MM, when detoxified traditionally, remains bioavailable without inducing cumulative toxicity.

This study also contributes to the broader discourse on evidence-based validation of traditional medicines, particularly in the context of WHO's Traditional Medicine Strategy (2014–2023), which underscores the importance of integrating pharmacovigilance and safety data into traditional medicine practice [25]. While centuries of empirical use lend credibility to MM, contemporary acceptance in regulated markets requires preclinical safety datasets such as the one presented here. The combined in vitro and in vivo evidence provides a scientific foundation for MM's safety, potentially facilitating its inclusion in integrative healthcare frameworks.

Nonetheless, certain limitations should be acknowledged. The present work was restricted to healthy animal models, which may not fully replicate disease-state pharmacodynamics or long-term safety under chronic administration. Additionally, heavy metal speciation and bioaccumulation analyses were not undertaken here but are critical to conclusively ruling out long-term toxicological risks, especially in the context of cumulative iron exposure [26-30]. Future studies should therefore incorporate chronic toxicity assessments, reproductive toxicity evaluations, and advanced toxicokinetic profiling, including tissue deposition studies using inductively coupled plasma mass spectrometry (ICP-MS). Moreover, clinical studies with rigorous safety monitoring will be essential to translate these findings into validated therapeutic guidelines.

5.Conclusion

The present study comprehensively evaluated the safety profile of *Mandoorathy Mathirai* (MM) through in vitro cytocompatibility assays on murine splenocytes, hepatocytes, and thymocytes, along with in vivo acute and sub-acute toxicity assessments in Wistar rats. The MTT assay results demonstrated high cell viability (>87%) across all tested concentrations, indicating minimal cytotoxicity and confirming the formulation's compatibility with immune, hepatic, and lymphoid cells. In vivo evaluations following OECD guidelines revealed no mortality, behavioural abnormalities, or significant deviations in body weight, food and water intake, organ weights, haematological indices, serum biochemical markers of hepatic and renal function, or lipid profile parameters. Histopathological analysis of vital organs, including liver, kidney, heart, and spleen, showed no structural alterations, further corroborating the absence of treatment-related toxicity. These findings collectively establish that MM, when prepared according to traditional purification practices, is safe for sub-acute oral administration at the tested doses, providing a robust scientific basis for its continued therapeutic use and future pharmacological investigations.

Acknowledgement

We wish to acknowledge my sincere thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India for their support.

6.References

1. Murali, M. & Essakkypandian, G. Preliminary screening of heavy metal analysis of a Siddha herbo-mineral formulation "Mandoora Mathirai" through a scientific technique ICP-OES. *Journal of Traditional and Integrative Medicine* 6 (2), 504–508 (2023).
2. Sarkar, P.K., Das, S., Prajapati, P.K. & Pandya, T.N. Safety and efficacy of herbo-mineral formulations in Siddha. *Ancient Science of Life* 31, 164–169 (2012).
3. Singh, R.H. *Ayurveda: The Science of Life*. (Chaukhambha Orientalia, Varanasi, 2011).
4. Saper, R.B. et al. Heavy metal content of Ayurvedic herbal medicine products. *JAMA* 300, 915–923 (2008).
5. Ernst, E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. *British Journal of Clinical Pharmacology* 54, 289–295 (2002).
6. OECD. *Guidelines for the Testing of Chemicals – Acute Oral Toxicity (OECD 423)*. (Organisation for Economic Co-operation and Development, Paris, 2001).
7. OECD. *Guidelines for the Testing of Chemicals – Repeated Dose 28-day Oral Toxicity Study in Rodents (OECD 407)*. (Organisation for Economic Co-operation and Development, Paris, 2008).
8. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65, 55–63 (1983).
9. Gad, S.C. *Animal Models in Toxicology*. (CRC Press, Boca Raton, 2014).
10. Hayes, A.W. & Kruger, C.L. *Hayes' Principles and Methods of Toxicology*, 7th edn. (CRC Press, Boca Raton, 2022).
11. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65, 55–63 (1983).
12. Freshney, R.I. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*, 8th edn. (Wiley-Blackwell, Hoboken, 2021).
13. Seglen, P.O. Preparation of isolated rat liver cells. *Methods in Cell Biology* 13, 29–83 (1976).
14. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K. & Gartner, F.H. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry* 150, 76–85 (1985).
15. Parasuraman, S. Toxicological screening. *Journal of Pharmacology & Pharmacotherapeutics* 2, 74–79 (2011).
16. Feldman, B.F., Zinkl, J.G. & Jain, N.C. *Schalm's Veterinary Hematology*, 5th edn. (Lippincott Williams & Wilkins, Philadelphia, 2000).

17. Prophet, E.B., Mills, B., Arrington, J.B. & Sobin, L.H. *Laboratory Methods in Histotechnology*. (Armed Forces Institute of Pathology, Washington DC, 1992).
18. Kiernan, J.A. *Histological and Histochemical Methods: Theory and Practice*, 5th edn. (Scion Publishing, Oxfordshire, 2015).
19. Tripathi, M., Singh, R. K., Prasad, G. & Kumar, V. Safety evaluation of traditionally purified iron oxide-based formulations: A cytotoxicity and oxidative stress perspective. *Journal of Ethnopharmacology* 274, 114045 (2021).
20. Giknis, M. L. A. & Clifford, C. B. *Comparative Haematology in Laboratory Animals*. Charles River Laboratories, Wilmington, MA (2020).
21. Shanmugapriya P , Sivaraman D, Christian GJ, Rajalakshmi K, Jeeva Gladys R .Hepatoprotective Potential of Novel Siddha Formulation Santha Santhrodhaya Mathirai against Paracetamol and Methotrexate Induced Hepatotoxicity in Chang liver Cell line Model. Journal of Pharmaceutical Sciences and Research (JPSR).2019;11(6): 2379-2384.
22. Sundaram, R., Murugesan, S., Anbarasu, S. & Ramesh, M. Toxicological assessment of herbo-mineral preparations in rodents: histopathological and biochemical perspectives. *BMC Complementary Medicine and Therapies* 20, 74 (2020).
23. Li, A. P. In vitro cytotoxicity assays for predicting in vivo toxicity: technical challenges and regulatory considerations. *Toxicology In Vitro* 36, 213–219 (2016).
24. Anbu, J., Jesudas, L. L., Natarajan, S. & Venkatesan, P. Safety assessment of Siddha herbo-mineral preparations: role of purification in mitigating heavy metal toxicity. *Ancient Science of Life* 36, 143–150 (2017).
25. World Health Organization. *WHO Traditional Medicine Strategy 2014–2023*. World Health Organization, Geneva (2013).
26. Prabu, S. M., Shagirtha, K., Renugadevi, J. & Aruldhas, M. M. Impact of metal accumulation on histopathology of vital organs: implications for herbal medicine safety. *Environmental Toxicology and Pharmacology* 50, 27–35 (2017).
27. Sonitha S, Sivaraman D, Rani V. Acute and subacute toxicity profiles on Siddha drug Thulasi Ennai in wistar rats. J Phytopharmacol 2020; 9(6):403- 409.
28. S. Arul Jothi , K.Dhivyalakshmi , N. Anbu , D.Sivaraman. Systematic Toxicity Profiling of Siddha Formulation Thoothula Pazha Chooranam by Acute and 28-Day Subacute Toxicity Studies in Swiss albino mice. International Journal of Advanced Research in Biological Sciences. Int. J. Adv. Res. Biol. Sci. (2019). 6(10): 11-22
29. Lavanya , R.Thamiloviam , U.Chitra , N. Anbu , D. Sivaraman. Toxicological Investigation of Siddha Preparation Agasthiyar Elathy Chooranam in accordance with OECD guideline. International Journal of Advanced Research in Biological Sciences. Int. J. Adv. Res. Biol. Sci. (2019). 6(10): 35-45.
30. K. Dhivyalakshmi , S. Arul Jothi , N. Anbu , D. Sivaraman .Preclinical Safety Evaluation of Sarabendira Siddha Maruthuva Sudar Chooranam by Acute and Subacute repeated oral toxicity Studies in Rodents. International Journal of Current Research in Biology and Medicine. Int. J. Curr. Res. Biol. Med. (2019). 4(10): 1-12