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Acute and subacute toxicity evaluation of *mega sanjeevi chooranam* in rats

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Abstract:
Mega Sanjeevi Chooranam (MSC), a Siddha formulation indicated for Madhumegam (Diabetes mellitus), lacks validated safety data. Therefore, it is of interest to evaluate its preclinical safety in Wistar rats using acute and 28-day repeated oral toxicity models in accordance with OECD guidelines 423 and 407. MSC caused no mortality or treatment-related toxic effects up to 2000 mg/kg (acute) and 1800 mg/kg/day (subacute), with no significant alterations in clinical, hematological, biochemical, or histopathological parameters, confirming a favorable safety profile for further clinical evaluation.

Keywords: *Mega Sanjeevi Chooranam*; siddha medicine; OECD guidelines; madhumegam; diabetes mellitus (DM)

Background:
The Siddha system of medicine describes health as the equilibrium of three vital humors—Vaatham, Piththam and Kabam—and their imbalance are considered the basis of several chronic diseases including diabetes mellitus **[1]**. Diabetes mellitus is described in classical Siddha literature as a metabolic disorder characterized by excessive urination, fatigue, weight loss and progressive tissue depletion, which clinically corresponds to diabetes mellitus **[2]**. Diabetes mellitus is a major global health concern affecting millions of people worldwide. The prevalence of diabetes continues to increase due to population aging, sedentary lifestyles, dietary changes and genetic susceptibility **[3, 4]**. India currently has one of the largest diabetic populations globally and epidemiological studies report a steady rise in prevalence among adults **[5].** National surveys indicate that approximately 9–10% of the adult population in India is affected by diabetes, with a significant proportion remaining undiagnosed or inadequately controlled **[6]**. The increasing disease burden contributes to substantial morbidity, mortality and healthcare expenditure. Traditional and complementary medicine systems are widely used for the management of chronic metabolic disorders such as diabetes. The World Health Organization (WHO) recommends systematic scientific evaluation of traditional formulations to ensure their safety, efficacy and quality before clinical use **[7]**. Although herbal medicines have a long history of traditional use, concerns regarding contamination, adulteration and intrinsic toxicity highlight the need for proper toxicological assessment **[8]**. MSC, documented in *Sarabendrar Siddha Maruthuva Sudar*, is traditionally used in the management of Diabetes mellitus and related metabolic disorders **[9]**. However, validated experimental data regarding its safety profile remain limited **[10]**. Therefore, it is of interest to assess preclinical toxicity studies conducted in accordance with OECD guidelines, which are essential for establishing the safety of such formulations before wider therapeutic application **[11–13]**.

Materials and Methods:
Selection of the test drug:
The test drug MSC is one of the polyherbal formulations for diabetes mellitus, which is indicated in the Siddha literature "*Sarabendrar Siddha Maruthuva Sudar*".

Collection of raw drugs:
The raw drug was purchased in a Country shop, in Chennai and authenticated by Dr. KN Sunil Kumar, Research Officer/Sci-II and HOD, Department of Pharmacognosy, Siddha Central Research Institute, Ministry of Ayush, Arumbakkam, Chennai-106, after studying its anatomical structure. The authentication certificate number is 576.28072301-10.

Ingredients of *Mega Sanjeevi Chooranam:*
The ingredients are depicted in **Table 1**.

Table 1: Ingredients of *Mega Sanjeevi Chooranam*

S. No	The vernacular name of the ingredients	Botanical name	Quantity
1.	*Naaval Vithai Paruppu*	*Eugenia jambolana*	100 g
2.	*Vilamichaver*	*Plectranthus vettiveroides*	100 g
3.	*Vetiver*	*Vetiveria zizanioides*	100 g
4.	*Ilavangam*	*Syzygium aromaticum*	100 g
5.	*Akkarakaram*	*Anacyclus pyrethrum*	100 g
6.	*Kondraippoo*	*Cassia fistula*	100 g
7.	*Aavarampoo*	*Cassia auriculata*	100 g
8.	*Thetrankottai*	*Strychnos potatorum*	100 g
9.	*Sirukurinjan Samoolam*	*Gymnema sylvestre*	500 g
10.	*Seenthil Sarkarai*	*Tinospora cordifolia*	100 g

Purification of ingredients [14]:
Eugenia jambolana:
The seeds were removed from the shell, followed by drying and frying.

Plectranthus vettiveroides:
The root was shade-dried under sunlight exposure and subsequently fried.

Vetiveria zizanioides:
The root was shade- dried under sunlight exposure and subsequently fried.

Syzygium aromaticum:
The bud of the clove was removed.

Anacyclus pyrethrum:
The material was purified by drying in sunlight and frying.

Cassia fistula:

The flowers were freshly collected, dried under sunlight and subsequently fried.

Cassia auriculata:
The flowers were freshly collected, dried under sunlight and subsequently fried.

Strychnos potatorum:
The seeds were removed from the shell, followed by drying and frying.

Gymnema sylvestre:
The whole plant was collected and dried under sunlight, followed by frying.

Salt extract from:
Tinospora cordifolia:
Remove the outer papery layer of *Tinospora cordifolia* stems and cut them into small segments. Using a stone mortar, crush the cut stems thoroughly. Place the crushed material into a wide container, add sufficient water and stir well to mix. Let the mixture stand undisturbed overnight. The next day, take out the fibrous residues from the water and rinse them in the same vessel. Repeat the process of collecting, crushing and rinsing to extract maximum content. Allow the vessel to sit for 2 to 3 hours to enable the sediment to settle at the bottom. Carefully pour out the clear upper layer and gather the settled starch. Add a fresh quantity of water and if any fibrous impurities remain, remove them. Let the starch settle once more, decant the water gently and collect the purified starch. Spread it out under sunlight to dry completely and store it in a clean, dry container **[14]**.

Preparation of MSC:
All purified and fried ingredients, except *Seenthil Sarkarai*, were powdered separately. The prepared *Seenthil Sarkarai* was added to the powdered ingredients and mixed thoroughly. The final product was stored in an airtight container **[2]**.

Experimental animal sourcing and care:
Toxicological assessment of MSC was carried out in animal models following prior approval from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Approval No: NIS/IAEC 22/R02/16112021/05 dated 16.11.2021 and NIS/IAEC-25/R05/06112023/11 dated 06.11.2023). The study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the National Institute of Siddha, Chennai and conducted in the institute's animal house facility located in Chennai, Tamil Nadu, India. Wistar albino rats (Rattus norvegicus) of both sexes, weighing 140-160g, were used for the study. The selected female rats were nulliparous and non-pregnant **[15]**. The animals were procured from an authorized animal breeder at the animal laboratory of TANUVAS, Madhavaram, Chennai and housed at the National Institute of Siddha, Chennai. The rats were maintained in polypropylene cages under controlled environmental conditions with a temperature of 22 ± 2°C, relative humidity of 40–65% and a 12 h light/dark cycle. A 7-day acclimatization period was provided prior to the experiment. The animals had free access to standard feed and RO water *ad libitum*. The toxicity study was carried out in accordance with OECD guidelines **[11, 12]**.

Determination of animal dose level:
The Siddha text specifies that the therapeutic dose of MSC is 60mg. The doses were determined from human therapeutic doses based on surface area, utilizing the conversion table of Paget and Barnes **[10, 16]**. The doses were as follows: Low dose – 90mg/kg b. wt Mid dose - 450/kg b. wt, High dose - 900mg/ kg b.wt.

Acute oral toxicity study:
An acute oral toxicity study of MSC was conducted in accordance with OECD Guideline 423. Six healthy female Wistar albino rats, all nulliparous and non-pregnant, were selected for the study, as female animals are known to exhibit slightly higher sensitivity to toxic substances. The animals were randomly divided into two groups, each consisting of three rats. The animals were acclimatized to laboratory conditions for seven days prior to the experiment. All rats were fasted overnight before dosing, while water was provided *ad libitum*. Body weight of each animal was recorded before dosing and the test dose was calculated individually based on body weight. In the first step, three rats received a single oral dose of MSC at 300 mg/kg body weight through oral gavage using a suitable vehicle. The animals were observed continuously for the first 30 minutes after dosing and subsequently at 1, 2 and 4 hours, followed by observations at 24, 48 and 72 hours and once daily thereafter for a total period of 14 days. As no signs of toxicity or mortality were observed at this dose level, a second group of three rats was administered a single oral dose of 2000 mg/kg body weight of MSC following the same procedure and observation schedule. All animals were monitored for mortality, morbidity and clinical signs of toxicity, including changes in general behavior, gait, skin and fur, eyes and mucous membranes, salivation, tremors, convulsions, lethargy, diarrhoea, sleep and coma. Body weights were recorded on day 0, day 7 and day 14 of the study period. At the end of the 14-day observation period, all animals were euthanized using excess anaesthesia (Thiopental sodium, 1 mg/100 g body weight) and a gross necropsy was performed to assess any pathological changes in thoracic and abdominal organs. In case of mortality during the study period, a complete necropsy was conducted. The median lethal dose (LD_{50}) cut-off value for MSC was determined in accordance with the Globally Harmonized System (GHS) classification as described in Annex 2d of OECD Guideline 423 **[11, 12]**.

Subacute oral toxicity study:
A 28-day repeated-dose oral toxicity study of MSC was conducted in accordance with OECD Guideline 407, with doses selected based on traditional Siddha therapeutic recommendations. A total of forty healthy adult Wistar albino rats (20 males and 20 females), aged 6–8 weeks and weighing 150–180 g, were used for the study. The human therapeutic dose

of MSC (2000 mg twice daily) was converted to the equivalent rat dose based on body surface area, resulting in a low dose of 180 mg/kg/day. Based on this, the animals were randomly divided into four groups (n = 10; 5 males and 5 females per group): Group I – control (vehicle only), Group II – low dose (180 mg/kg/day), Group III – mid dose (900 mg/kg/day) and Group IV – high dose (1800 mg/kg/day). All treatments were administered once daily by oral gavage for 28 consecutive days. Animals were observed daily for clinical signs of toxicity and mortality. Body weights were recorded on Day 0, weekly thereafter and on the day of necropsy. Food intake was monitored weekly and mean consumption was calculated for each group. Clinical observations included general behavior, skin and fur condition, eyes and mucous membranes, respiration, motor activity, reflexes and signs of distress such as tremors, convulsions, lethargy, sleep, diarrhoea, or coma. Mortality and morbidity were checked twice daily. At the end of the treatment period, animals were fasted overnight and euthanized using carbon dioxide inhalation. Blood samples were collected by cardiac puncture for hematological and biochemical analyses. Hematological parameters included haemoglobin, RBC, WBC with differential count, haematocrit, MCV, MCH, MCHC and platelet indices. Biochemical markers such as glucose, lipid profile, urea, creatinine, bilirubin, SGOT, SGPT and ALP were assessed using automated analyzers. Following blood collection, gross necropsy was performed and major organs including brain, heart, lungs, liver, kidneys, spleen, stomach, intestines and reproductive organs were examined for lesions and weighed.

Relative organ weights were calculated using the Formula: (organ weight / body weight) × 100.

Histopathological examination was conducted on selected organs from control and high-dose animals showing altered biochemical markers. Tissues were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned at 4–5 µm and stained with haematoxylin and eosin. Microscopic evaluation was performed under a light microscope.

Statistical analysis:

Data were expressed as mean ± SEM and statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test, with $p < 0.05$ considered statistically significant.

Results and Discussion:

Body weight changes observed during the acute toxicity study in male rats are presented in **Table 2**, while body weight changes during the sub-acute toxicity study in female rats are shown in **Table 3**. Food intake of male and female rats is summarized in **Tables 4 and 5**, respectively. Water intake data for male and female rats are presented in **Tables 6 and 7**, respectively. The effect of MSC on relative organ weights of male and female rats following the 28-day repeated dose oral toxicity study is detailed in **Table 8**. Haematological parameters of male and female rats are presented in **Tables 9 and 10**, respectively. Biochemical parameters of male and female rats following the 28-day repeated dose oral toxicity study are summarized in **Tables 11 and 12**, respectively. Additional biochemical parameters of female rats following the 28-day repeated dose oral toxicity study are presented in **Table 13.** Histopathological findings of control and high-dose treated male rats are illustrated in **Figures 1 and 2**, showing the brain, heart, kidney, liver, lungs and spleen. Histopathological observations of control and treated female rats, including the brain, heart, kidney, liver, lungs, spleen, stomach, uterus and ovary, are depicted in **Figures 3 and 4**.

Table 2: Body weight changes in acute oral toxicity studys

Dose/Day	0th Day	7th Day	14th Day
300 mg/kg b. wt	146.50±2.50	157.00±4.51	164.25±3.01
2000 mg/kg b. wt	156.75±2.06	168.50±3.30	177.50±3.30

Results are shown as Mean±SEM. Group size was 3 for all groups

Table 3: Body weight changes in male rats

Dose/Day	Day 1	Day 7	Day 14	Day 21	Day 28
Control	190.4±8.73	252.00±13.17	261.40±13.12	271.80±16.29	289.40±17.53
Low Dose	188.20±4.43	239.60±14.22	253.40±14.51	269.80±12.33	288.00±8.18
Mid Dose	187.00±9.24	246.40±16.08	269.80±33.61	255.60±18.18	273.40±20.91
High Dose	188.00±8.63	233.20±9.32	241.40±9.56	250.20±9.33	267.60±9.66

Table 4: Body weight changes – female rats

Dose/Day	Day 1	Day 7	Day 14	Day 21	Day 28
Control	164.40±6.99	187.20±5.68	189.00±6.36	190.00±6.50	193.00±3.83
Low Dose	162.40±5.61	182.60±5.31	186.40±5.75	194.60±6.59	195.80±8.63
Mid Dose	160.00±6.14	184.80±9.09	190.80±10.61	194.40±10.57	198.40±10.63
High Dose	156.60±7.73	181.60±2.79	181.00±3.11	182.20±3.43	192.00±3.81

Results are shown as Mean±SEM. Group size was 5 for all groups

Table 5: Food Intake – Male rats

Dose/Day	Day 1	Day 7	Day 14	Day 21	Day 28
Control	65.43±1.07	69.00±2.01	71.57±1.95	82.00±2.06	92.43±1.04
Low Dose	63.00±0.65	68.57±2.38	76.14±1.70	84.14±1.62	95.86±0.86
Mid Dose	61.29±1.76	65.71±1.97	71.86±2.19	80.00±2.29	91.57±1.91
High Dose	60.65±1.67	64.57±2.75	70.00±2.27	77.14±2.40	85.86±2.81

Results are shown as Mean±SEM. Group size was 5 for all groups

Table 6: Food Intake – Female rats

Dose/Day	Day 1	Day 7	Day 14	Day 21	Day 28
Control	42.00±0.35	45.00±0.77	48.00±0.40	52.00±0.32	58.00±0.54
Low Dose	43.00±0.84	45.00±0.55	48.00±0.55	53.00±0.32	55.00±0.32
Mid Dose	42.00±0.22	46.00±0.71	49.00±0.65	52.00±0.50	54.00±0.19
High Dose	40.00±0.69	44.00±0.50	48.00±0.27	54.00±0.71	57.00±0.32

Results are shown as Mean±SEM. Group size was 5 for all groups

Table 7: Water Intake – Male rats

Dose/Day	Day 1	Day 7	Day 14	Day 21	Day 28
Control	82.00±0.57	87.00±0.22	96.00±0.61	105.00±0.27	114.00±0.55
Low Dose	80.00±1.11	90.00±0.52	98.00±0.42	103.00±0.77	108.00±0.55
Mid Dose	85.00±0.35	86.00±0.74	100.00±0.71	104.00±0.84	110.00±1.82

High Dose	84.00±1.17	82.00±1.60	102.00±1.14	110.00±1.92	116.00±0.84

Results are shown as Mean±SEM. Group size was 5 for all groups

Table 8: Water intake – female rats

Dose/Day	Day 1	Day 7	Day 14	Day 21	Day 28
Control	68.00±1.64	70.00±2.02	76.00±1.92	80.00±0.84	86.00±1.38
Low Dose	66.00±1.05	72.00±1.86	77.00±1.30	82.00±1.90	87.00±1.30
Mid Dose	63.00±1.22	71.00±1.29	75.00±0.95	83.00±1.14	88.00±1.14
High Dose	69.00±1.00	74.00±0.91	79.00±1.35	86.00±0.84	90.00±0.71

Results are shown as Mean±SEM. Group size was 5 for all groups

Table 9: Effect of MSC on Relative Organ Weight (g) in 28 Day Repeated Dose Oral toxicity study in male and female rats

Organ	GroupI-Control		GroupII-LowDose		GroupIII-MidDose		GroupIV-HighDose	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver	12.76±1.03	7.80±0.19	12.02±0.81	8.58±0.74	10.79±1.20	8.08± 0.52	9.98±0.56	7.74±0.20
Lungs	1.70±0.22	1.45±0.09	2.01±0.20	1.25±0.06	1.76±0.12	1.42±0.09	2.20±0.26	1.74±0.09
Heart	1.09±0.12	0.77±0.04	0.95±0.02	0.74±0.04	0.96±0.09	0.93±0.18	0.92±0.02	0.75±0.03
Spleen	0.77±0.11	0.72±0.01	1.31±0.13	0.76±0.05	0.67±0.05	0.71±0.07	1.08±0.15	0.75±0.05
Brain	1.71±0.08	1.71±0.05	1.88±0.17	1.71±0.06	1.75±0.05	1.70±0.01	1.80±0.02	1.76±0.02
Stomach	1.76±0.13	1.40±0.06	1.77±0.13	1.52±0.11	1.69±0.12	1.45±0.13	1.74±0.08	1.57±0.04
Kidney	3.10±0.42	1.93±0.16	2.39±0.08	1.80±0.07	2.55±0.34	1.75±0.09	2.27±0.14	1.71±0.05
Testis& Epididymis/ Uterus& Ovary	4.55±0.18	0.57±0.03	4.57±0.23	0.72±0.06	4.22±0.53	0.64±0.06	4.29±0.32	0.64±0.05

All values are expressed as Mean ± SEM, N=5

Table 10: Effect of MSC on Haematological parameters of male rates in 28 days repeated dose oral toxicity study

Groups	**RBC**	**WBC**	**HCT**	**HGB (g/dL)**	**Lym**	**MCH**	**MCHC**	**MCV**	**Mon # (10^3/uL)**	**MPV (fL)**
	(10^6/UL)	**(10^3/uL)**	**(%)**		**(10^3/uL)**	**(pg)**	**(g/dL)**	**(fL)**		
Control	7.87±0.20	16.11±4.85	67.28±2.15	14.72±0.38	12.25±4.12	18.74±0.23	21.92±0.17	85.46±1.08	1.55±0.44	9.22±0.23
Low Dose	7.43±0.17	23.08±1.27	62.92±1.73	13.80±0.36	18.94±1.07	18.56±0.22	21.92±0.09	84.74±0.99	1.90±0.10	8.46±0.21[a]
Mid Dose	7.32±0.37	9.83±0.43	63.04±3.20	14.10±0.53	7.46±0.44	19.32±0.54	22.42±0.62	86.14±0.70	0.74±0.08	9.16±0.07
High Dose	7.47±0.26	18.77±3.53	63.48±1.93	13.95±0.52	14.63±3.32	18.73±0.20	22.08±0.09	85.04±0.51	1.86±0.31	8.70±0.23

Results are shown as Mean ± SEM. Group size was 5 for all groups. a = p<0.05, compared to control group.

Table 11: Effect of MSC on Haematological parameters of male rates in 28 days repeated dose oral toxicity study

Groups	**RBC**	**WBC**	**HCT**	**HGB (g/dL)**	**Lym**	**MCH**	**MCHC**	**MCV**	**Mon # (10^3/uL)**	**MPV (fL)**
	(10^6/UL)	(10^3/uL)	(%)		(10^3/uL)	(pg)	(g/dL)	(fL)		
Control	6.87±0.25	13.88±1.47	60.82±2.13	13.08±0.39	11.63±1.37	19.06±0.20	21.54±0.12	88.48±0.49	1.12±0.17	9.22±0.12
Low Dose	7.27±0.16	18.92±0.98	62.94±1.15	13.70±0.27	15.55±1.10	18.84±0.22	21.74±0.07	86.60±1.06	1.70±0.27	9.16±0.17
Mid Dose	6.28±0.79	14.75±3.58	55.50±6.81	11.90±1.52	11.42±2.76	18.88±0.17	21.32±0.23	88.60±0.69	1.63±0.45	8.94±0.02
High Dose	7.48±0.09	16.70±2.63	64.34±0.81	13.92±0.19	13.79±2.17	18.68±0.12	21.68±0.12	86.04±0.65	1.62±0.44	8.76±0.17

Results are shown as Mean ± SEM. Group size was 5 for all groups.

Table 12: Effect of MSC Biochemical Parameters of Male in 28 days repeated dose oral toxicity study

Dose/Day	**Glucose**	**Urea**	**Creatinine**	**T. Chol**	**HDL**	**LDL**	**TGL**	**SGOT**	**SGPT**	**T. Protein**	**Albumin**	**Uric Acid**
Control	222.03±45.77	28.23±3.56	0.55±0.12	25.27±3.01	50.61±12.71	81.34±9.17	88.54±19.83	69.83±10.84	31.51±3.99	4.98±2.23	2.84±0.53	9.71±1.05
Low Dose	190.10±40.34	27.93±2.78	0.53±0.08	43.32±19.12	47.49±8.05	79.26±11.87	60.26±4.43	61.95±5.75	36.45±1.62	6.35±1.35	2.21±0.31	4.22±0.94
Mid Dose	159.86±23.77	21.54±2.56	0.41±0.02	50.60±5.62	73.31±11.41	111.49±26.24	81.34±21.64	69.45±7.97	36.17±2.11	8.39±1.67	2.64±0.36	4.25±0.90
High Dose	180.84±24.73	23.84±2.82	0.48±0.03	99.94±13.32	94.33±8.68	137.32±13.16	105.77±12.74	83.51±5.65	38.56±1.26	9.46±1.35	2.97±0.44	7.07±0.74

Results are shown as Mean±SEM. Group size was 5 for all groups

Table 13: Effect of MSC Biochemical parameters of female in 28 days repeated dose oral toxicity study

Dose/Day	Glucose	Urea	Creatinine	T.Chol	HDL	LDL	TGL	SGOT	SGPT	T. Protein	Albumin	Uric Acid
Control	296.75±49.56	31.82±1.95	0.62±0.06	76.64±14.40	95.83±12.22	125.58±10.67	87.6±13.25	129.81±27.76	61.42±4.90	9.42±0.58	5.36±0.43	7.22±0.58
Low Dose	275.86±24.15	27.41±2.15	0.57±0.05	178.58±25.13	81.76±9.80	133.16±11.08	87.47±6.95	153.76±30.15	61.32±1.73	9.58±0.96	3.31±0.09	6.45±0.78
Mid Dose	253.62±42.07	29.68±1.07	0.63±0.04	83.00±11.38	85.51±6.65	17.69±1.31	78.25±14.60	121.16±8.71	54.70±4.24	6.25±0.73	2.61±0.42	7.35±0.88
High Dose	337.42±63.19	20.24±2.76	0.61±0.02	92.19±10.63	80.14±3.49	24.31±2.16	57.38±8.94	102.63±12.65	52.26±3.44	6.44±0.99	2.47±0.24	7.76±1.03

Results are shown as Mean±SEM. Group size was 5 for all groups

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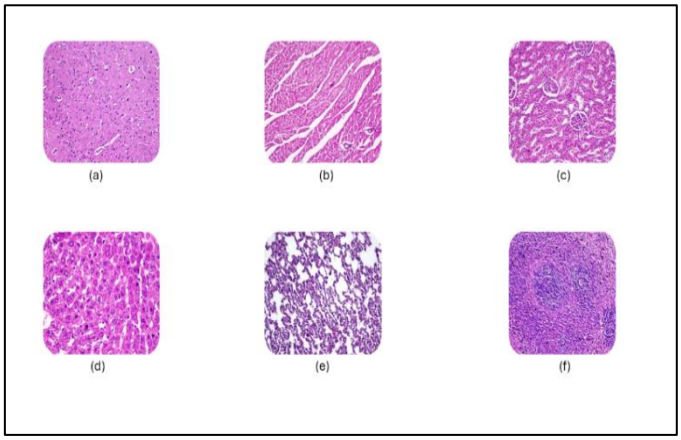

Figure 1: Histopathology of Control male rats (28-Day Oral Toxicity Study of MSC) a) Brain (b) Heart (c) Kidney (d) Liver (e) Lungs (f) Spleen

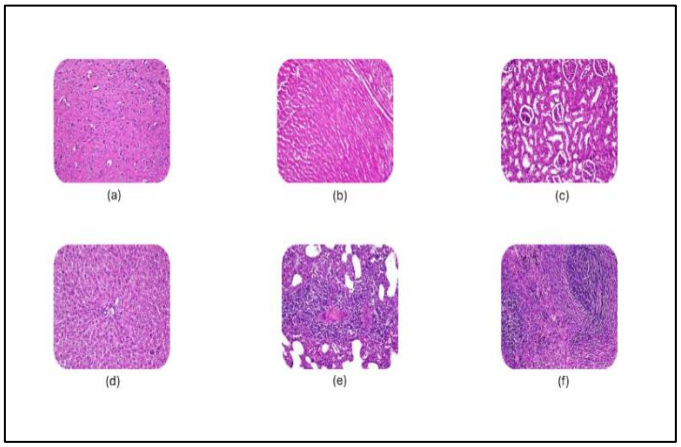

Figure 2: Histopathology of High dose male rats (28-Day Oral Toxicity Study of MSC) a) Brain (b) Heart (c) Kidney (d) Liver (e) Lungs (f) Spleen

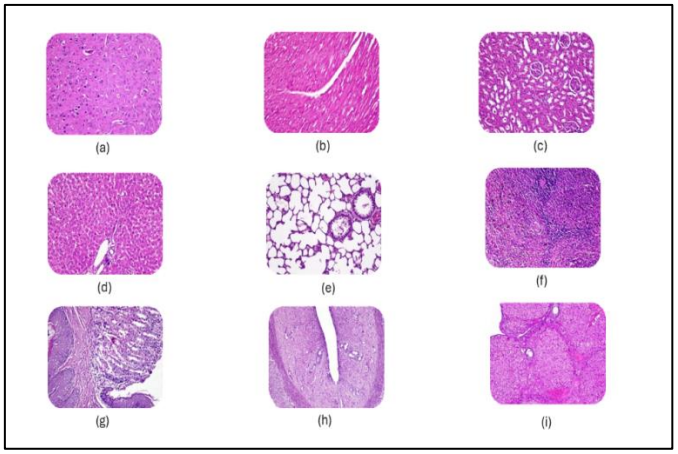

Figure 3: Histopathology of control female rats (28-Day Oral Toxicity Study of MSC) a) Brain (b) Heart (c) Kidney (d) Liver (e) Lungs (f) Spleen g) Stomach h) Uterus i) Ovary

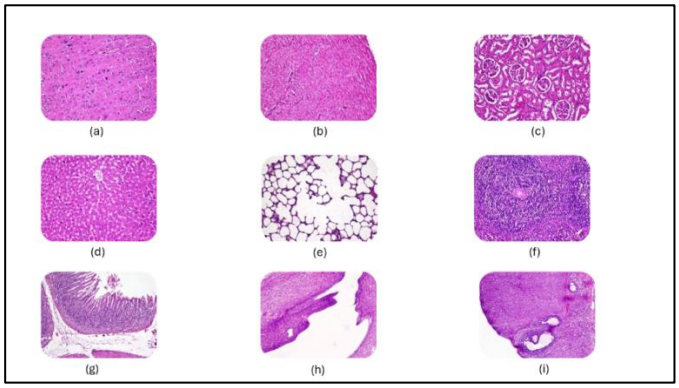

Figure 4: Histopathology of control female rats (28-Day Oral Toxicity Study of MSC) a) Brain (b) Heart (c) Kidney (d) Liver (e) Lungs (f) Spleen g) Stomach h) Uterus i) Ovary

The present study was undertaken to scientifically evaluate the safety profile of MSC, a classical Siddha polyherbal formulation traditionally used in the management of Diabetes mellitus, through acute and 28-day repeated dose oral toxicity studies in Wistar albino rats, following OECD guidelines 423 and 407. Although MSC has been used in traditional practice for several decades, systematic toxicological data were lacking, necessitating preclinical safety validation. In the acute oral toxicity study, administration of MSC up to 2000 mg/kg body weight did not produce any mortality or observable clinical signs of toxicity. Normal behavior, feeding patterns and progressive body weight gain observed throughout the 14-day observation period indicate good tolerability of the formulation. According to the Globally Harmonized System (GHS), the absence of mortality at this dose suggests that MSC falls under category 5, a low-toxicity category, with an LD_{50} cut-off value greater than 2000 mg/kg. Acute toxicity data are of limited significance since cumulative toxic effects can occur even at very low doses. Hence, multiple dose studies are usually helpful in evaluating the safety profile of polyherbal formulation **[17]**. 28-day repeated oral toxicity study was therefore carried out. Repeated-dose toxicity studies were performed to assess the potential adverse effects of the test drug MSC following repeated administration. These studies were designed to identify possible health risks associated with short-term repeated exposure, to evaluate the likelihood of cumulative toxic effects and to determine the dose level at which no observable adverse effects occur. In the 28-day repeated dose oral toxicity study, MSC did not induce treatment-related adverse effects on general health, behavior, food and water intake, or survival in both male and female rats. Assessment of food consumption was an essential component of the safety evaluation of the therapeutic product, as adequate nutrient intake is critical for maintaining the normal physiological status of the animals. Proper nutrition ensures that the observed responses are attributable to the test drug itself rather than confounding effects arising from inadequate or imbalanced nutritional conditions **[18]**. The gradual increase in body weight observed across all dose groups was comparable to that of the control group, indicating that MSC did not interfere

with normal growth or metabolic activity. Maintenance of normal feed and water consumption further supports the absence of systemic toxicity or distress. Relative organ weight is a sensitive indicator of target organ toxicity. In the present study, relative organ weights of major organs, including liver, kidneys, heart, lungs, spleen, brain and reproductive organs did not show any significant or dose-dependent alterations. This suggests that repeated exposure to MSC did not cause organ hypertrophy or atrophy, reinforcing its systemic safety. Haematological parameters serve as important indicators of bone marrow function, immune status and physiological balance **[19]**. Although minor statistical variations were observed in certain haematological indices, such as MPV and differential leukocyte counts, these changes remained within normal physiological limits and lacked dose dependency. Therefore, they are considered incidental rather than toxicologically relevant. Importantly, no evidence of anemia, leukocytosis, or hematopoietic suppression was observed, indicating that MSC does not adversely affect hematological homeostasis. Biochemical parameters reflecting hepatic, renal and metabolic function largely remained within normal limits. The absence of significant elevations in serum transaminases, urea and creatinine suggests that MSC does not exert hepatotoxic or nephrotoxic effects even at higher dose levels. Interestingly, reductions in total cholesterol and LDL levels, along with increases in HDL in certain dose groups, may indicate a favorable metabolic influence rather than toxicity. However, these observations require further pharmacodynamic investigation and should not be overinterpreted within a toxicity framework. Histopathological examination of vital organs from control and high-dose groups revealed normal cellular architecture without evidence of inflammation, necrosis, degeneration, or pathological lesions. Preservation of normal histomorphology of the liver, kidneys, heart, lungs, spleen, brain and reproductive organs strongly corroborates the biochemical and organ weight findings, confirming the absence of target organ toxicity. Thus, the findings of the present study demonstrate that MSC is non-toxic when administered orally in rats, with a no-observed-adverse-effect level (NOAEL) above 1800 mg/kg/day for 28 days. The results provide scientific support for the traditional use of MSC and establish a preclinical safety foundation for its continued therapeutic application. Nevertheless, further long-term toxicity, reproductive toxicity and clinical safety studies are recommended to strengthen its translational relevance and regulatory acceptance.

Conclusion:

The acute and subacute toxicity studies of MSC in Wistar albino rats (*Rattus norvegicus*) showed no significant alterations in vital organ morphology, hematological parameters, or biochemical profiles. The absence of treatment-related toxicological effects supports the safety of MSC and is consistent with its long-standing use in Siddha medicine. These findings provide scientific evidence for its safe use as per traditional guidelines; however, further clinical studies are necessary to confirm its safety and therapeutic efficacy in humans.

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