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ORIGINAL RESEARCH ARTICLE

Acute and Sub Acute Toxicity Study on Siddha Drug *Mandoora chooranam*

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ABSTRACT

Mandoora chooranam(MC) has been used for treatment of Iron deficiency anaemia (IDA)which is a herbo-mineral formulation. As a mandate,steps were taken to evaluate safety profile of MC in rats following OECD guidelines. Acute toxicity studies were done on female wistar albino rat under OECD guidelines 423 and 28 days repeated oral toxicity studies were done on both sexof wistar albino rats under OECD guidelines 407.Acute toxicity studies, MCwas administered single oral dose and observed for 14 days. In Acute toxicity studies no toxic effects were observed upto the dose level of 2000mg/kg body weight.Sub-acute toxicity studies were carried in four different groups in which MC was administrated orally to rats once daily for 28 days in various dosesranging from 40, 200,400 mg/kg for rat respectively. Detailed hematological, biochemical, necropsy and histopathological evaluation of organs was performed for all animals. No toxic effect was observed upto 400mg/kg in sub-acute toxicity studies of *Mandoora chooranam.*

Keywords: *Mandoora Chooranam*, Iron Deficiency Anaemia, IDA, Acute toxicity, Sub-acute toxicity.

INTRODUCTION

The interventional drug *Mandoora chooranam* (MC) has been quoted by Sarabendira vaidya muraigal Paandu Kamalai Roga Sikitchai for the treatment of Paandu (IDA) [1].Iron Deficiency Anaemia (IDA) is one of the most widespread diseases all over the world, incidence is high in reproductive age of women and children [2].Among worldwide distribution of anaemia approximately half of them are due to Iron deficiency anaemia. Women are affected more than men and it's widely prevalence is about700-800 million people in under developed countries and 60 – 70 million in developed countries [3].

It is the most important cause of microcytic hypochromic anaemia in which all the three red cell indices (MCV, MCH and MCHC) are reduced and occurs due to defective haemoglobin synthesis [4]. Major causes of iron deficiency are chronic blood loss, increased iron requirement, iron malabsorption and inadequate dietary intake [5].

There are many alternative treatments available that can treat the IDA. Herbs and minerals have been in use since long time to treat various diseases [6]. However, many issues related to a lack of scientific evidence about the efficacy and safeties of the drugs remain unresolved [7,8]. The Pre-clinical toxicity studies were essential for determining a safe dose for human trials[9].Consequently an effort was made to evaluate acute and sub-acute toxicity of herbo-mineral siddha formulation of *Mandoora chooranam* in laboratory animals.

MATERIALS & METHODS

Preparation of the *Mandoora chooranam*:

a)**Ingredients:** Purified *Mandooram*(Ferroso ferric oxide), Kodiveli Root(*Plumbago zeylanica*), Thippili(*Piper longum*), Kadukkai Thol(*Terminalia chebula*), Nellimulli(*Emblica officinalis*), Chevuiyam(*Pipernigrum*), Maramanjal(*Coccinium fenestratum*), Chukku(*Zingiber officinale*), Milagu(*Pipernigrum*), Thandrikkai(*Terminalia bellarica*), Kandthippili (*Piper longum*),

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Vaividangam(*Embelia ribes*), Devadharu(*Cedrus deodara*).

b) **Procedure:**

Mandooram is purified using cow's urine and made into powder form. Other raw drugs are purified and powdered.

Equal amount of mandooram powder and equal amount of raw drugs powder are mixed well, and collected [1].

Animals:

Rats of either sex weighing 150-200gm were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai and maintained in the animal laboratory of Sairam Advanced Centre for Research.The animals were used with the approval of the Institute animal ethics committee (IAEC) of Sairam Advanced Centre for Research, Chennai approval no. (1545/PO/a11/CPCSEA/1-4/2013). All the animals were kept under standard environmental condition (23±2°C), standard light cycle (12 h light, 12 h dark). The animals had free access to water and standard pellet diet.

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute Toxicity Study-OECD423 guidelines [10,11]:

Acute oral toxicity test for the *Mandoora chooranam* was carried out as per OECD Guidelines 423.Female wistar albino rats were fasted over night prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days, to observe any death or changes in general behavior and other physiological activities. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern.

Sub-Acute Toxicity –OECD 407 Guidelines [12]:

In a 28-days, sub-acute toxicity study, ten rats (Five Male and Five Female)were in each group divided into four groups. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the Mandoora chooranam(p.o.) for 28 days at a dose of 40mg/kg(x), 200mg/kg(5x) and 400mg/kg(10x) respectively.The weight of each rat was recorded on day 0 and weekly throughout the course of the study , food and water consumption per rat was calculated .

At the end of the 28 days they were fasted overnight, each animal was anaesthetized with ether, following which they were then dissected and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters

RESULTS

Acute oral toxicity

Table 1: Dose finding experiment and its behavioral Signs of Toxicity

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

Acute oral toxicity 28-day repeated dose study

Table2: Bodyweight (g) changes of albino rats exposed to Mandoora Chooranam for 28 days

Dose (mg/kg/day)	*Days*				
	1	**7**	**14**	**21**	**28**
Control	122.37±3.21	124.14±4.09	118.21±2.17	127.21±5.11*	133.32±1.89*
40	124.18 ±3.12	125.24 ±2.35	127.31 ±4.21	128.27 ±3.65	134.26 ±5.24*
200	128.22±6.37	129.41±8.26	130±24.25	132.32±8.40	135.27±10.14
400	126.24±09.10	128.77±12.11	130.64±13.67	131.08±7.16*	132.16±8.46*

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

Table 3: Food (g/day) intake of albino rats exposed to Mandoora Chooranam for 28 days

Dose (mg/kg/day)	**Days**				
	1	**7**	**14**	**21**	**28**
Control	38.25±3.10	36.18±2.78	39.36±2.10	36.14±2.80	39.20±2.12
40	37.35±2.44	37.17±2.18	38.24±2.30	38.23±2.72	38.12±3.00
200	39.60±2.41	39.40±3.12	38.10±2.44	40.77±2.55	41.46±1.44
400	41.10±2.07	41.04±2.12	42.10±2.45	42.2 6±3.08	42.56±2.05

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

Table 4: Water (ml/day) intake of albino rats exposed to Mandoora Chooranam for 28 days

Dose (mg/kg/day)	**Day**				
	1	**7**	**14**	**21**	**28**
Control	44.2468±3.00	43.32 ±3.22	45.21 ±3.14	43.32 ±2.54	42.10±2.96
40	46.12±2.45	47.24±3.24	48.24±2.60	49.23 ±2.74	50.35 ±3.20
200	48.35 ±1.24	48.60 ±2.99	48.22 ±2.55	48.05±1.23	47.07±3.27
400	47.07 ±1.34	47.15 ±1.45	48.24 ±1.02	49.23 ±1.77	50.24 ±2.44

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

Hematological and blood biochemical analyses:

At the end of the study, all animals were kept fasted for 18 h and then anesthetized with anesthetic ether on the 29th day. Blood samples for hematological and blood chemical analyses were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count (**Table 5**) (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin)by semi-automated hematology analyzer.

Table 5: Effect of Mandoora Chooranam on Haematological parameters after 28 days

Parameter	Control	40 mg/kg	200 mg/kg	400 mg/kg
Red blood cell (mm^3)	7.51±0.16	6.87±0.31	6.52±0.22	6.25±0.11
HB (%)	15.60±0.19	15.34±0.16	15.64±0.29	15.46±0.26
Leukocyte ($x10^6$/mL)	10124±126.51	10252±286.05	10401±246.35	10380±264.14
Platelets/ul	1335±17.32	1234±34.34	1245±42.27	1246±17.23
MCV (gl)	58.32±2.21	57.16±1.56	57.42±1.17	56.04±1.28
DLC (%)				
N	4.38±1.42	5.50 ±3.12	4.48±1.24	5.44±1.01
L	91.12±4.12	91.42±3.21	91.12±3.41	91.14±2.74
M	2.11±1.23	2.40±1.445	2.24±0.80	2.75±0.63
E	1.12±0.22	1.24±0.30	2.10±0.28	1.98±0.24
B	0	0	0	0
ESR(mm)	1±00	1±00	1±00	1±00
PCV	48.20±2.44	46.32±3.10	46.14±2.65	47.30±2.14
MCH pg	18.38±1.52	17.41±0.88	18.46±00.65	18.78±0.21
MCHC g/dl	30.67±1.23	31.04±0.85	31.18±2.63	30.69±1.35

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

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure (**Table 6, 7, 8 & 9**).

Table 6: Effect of Mandoora Chooranam Hepatic parameters

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Total Bilirubin (mg/dL)	0.205±1.01	0.206±0.12	0.207±0.14	0.208±1.02
Bilirubin direct (mg/dL)	0.1±0.1 7	0.1±0.1 5	0.1±0.1 9	0.1±0.14
Bilirubin indirect(mg/dL)	0.1±00	0.1±00	0.1±00	0.1±00
SGOT (U/L)	166.44±3.24	164.78±2.52	162.42±3.45*	159.14±2.12*
SGPT(U/L)	45.4±2.14	44.4±2.28	44.00±2.15	45.62±4.13
Total Protein(g/dl)	10.62±1.30	10.16±0.30	9.42±0.27	10.11±0.46
Albumin(g/dl)	3.31±0.24	3.24±0.22	3.35±0.20	3.24±0.15
Globulin(g/dl)	6.12±0.24	5.24±0.30	6.77±0.34	6.48±0.10
A/G Ratio(g/dl)	0.55±0.21	0.54±0.21	0.65±0.41	0.63±0.5 0
GGT(U/L)	7.4±0	7.2±0	7.1±0	7.1±0

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

Table 7: Effect of Mandoora Chooranam Renal parameters

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Urea(mg/dL)	64.24±3.11	62.26±5.21	64.12±1.25	68.44±1.31
Creatinine (mg/dL)	0.82±0.16	0.83±0.14	0.81±0.23	0.82±0.22
Uric acid (mg/dL)	1.6±0.21	1.6±0.23	1.6±0.22	1.56±0.26
Na m.mol	138.12±3.14	137.4±3.41	138.12±3.14	139.18±2.01
K m.mol	20.50±2.34	19.51±2.18	20.10±2.28	20.23±2.20
Cl m.mol	99.24±2.11	100.10±4.24	98.28±3.62	102.20±5.58*

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

Table 8: Effect of Mandoora Chooranam on Lipid profile

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Total cholestrol(mg/dL)	41.24±1.35	40.12±1.02	43.22±2.12	45.15±3.12
HDL(mg/dL)	12.40±1.45	12.29±1.36	12.27±1.20	13.19±3.00
LDL(mg/dL)	38.18±2.88	42.11±3.18	37.14±2.47	35.14±1.88
VLDL(mg/dl)	16.45±2.46	16.24±2.10	16.36±1.66	14.10±1.13
Triglycerides (mg/dl)	82.14±1.23	81.22±1.35	81.11±1.110	83.21±1.36
TC/HDL ratio (g/dl)	3.37±2.21	3.29±1.26	3.47±2.27	3.54±4.28
Blood glucose(mg/dl)	110.16±8.62	112.0±3.33	112.37±4.12	112.4±2.58

Table 9: Effect of Mandoora Chooranam on Urine parameters

Parameters	Control	40 mg/kg	200 mg/kg	400 mg/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Turbid	Cloudy	Slightly turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	>7.2	>8.0	>8.0	>9.0
Protein	Nil	2+	2+	1+
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	+ve	+ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Abnormal	Abnormal	Abnormal
Pus cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	1-cell/HPF	Nil	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

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

Necropsy:

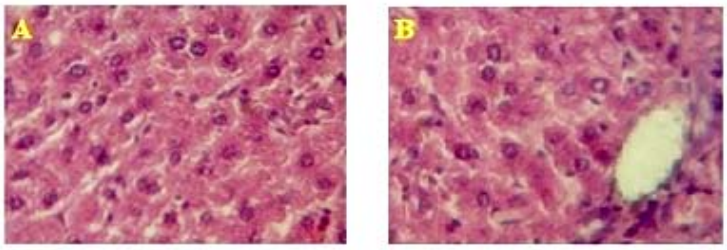
All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus and testes/ovaries were recorded (**Table 10**) . Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosinand were examined microscopically (**Panel 1, 2, 3, & 4**).

Table 10: Effect of Mandoora Chooranam on Organ weight

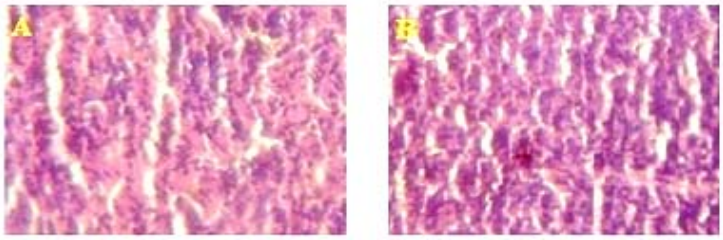
Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Liver (g)	5.24±0.14	4.45±0.21	4.75±0.20	4.23±0.22
Heart (g)	0.70±0.05	0.67±0.02	0.65±0.05	0.66±0.02
Lung (g)	1.78±0.25	1.53±0.21	1.45±0.22	1.74±0.10
Spleen (g)	0.74±0.07	0.62±0.05	0.69±0.05	0.66±0.04

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	1.42±0.17	1.44±0.16	1.44±0.18
Kidney (g)	0.70±0.05	0.71±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

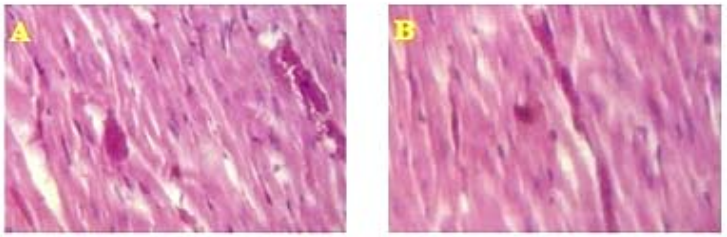
Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01 *vs control* N=10.



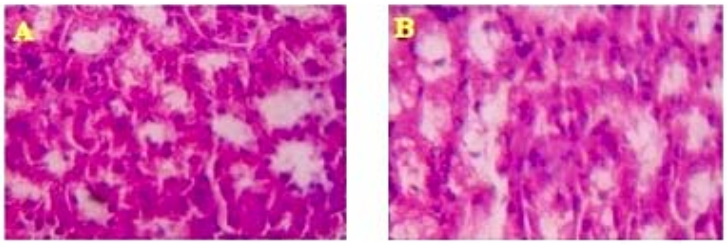
Panel 1: Light photomicrography of liver of a control rat
Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in hepatocytes, sinusoids.



Panel 2: Light photomicrography of Spleen of a control rat
Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in trabeculae, capsule.



Panel 3: Light photomicrography of Heart of a control rat
Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in nuclei of
Myocytes, myocardium



Panel 4: Light photomicrography of Kidney of a control rat
Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in glomeruli, Bowman's capsule, capillaries.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova followed by dunnet't' test using a computer software programme -INSTAT-V3 version.

DISCUSSION

The results of acute toxicity study of *Mandoora Chooranam* revealed no mortality, abnormal signs and behavioral changes in rats at the dose of 2000 mg kg-1 body weight administered orally. All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. Animals from treated groups show comparable body weight gain that of controls throughout the dosing period of 28 days. Food and water consumption of control and treated animals was found to be comparable during the dosing period of 28days.

The results of haematological investigations revealed no significant changes in the values when compared with those of respective controls. Among the differential count of WBC, only the Eosinophil's count was slightly increased at the dosage of 40 mg/kg and 200 mg/kg. This might be occurred due to stress.Results of Biochemical investigations conducted on days 29, Urea, SGOT,SGOT, Bilirubin were within the limits. LDL level was elevated in animals of 40 mg/kg dose group (P<0.05) and at the dosage of 400mg/kg, total cholesterol level was slightly increased but these were within the normal limits.

The other cardio vascular risk markers were also within normal limits.

Urine analysis data of control group and treated group of animals determined in week 4 did not reveal major abnormalities rather than transparency, pH and deposits. Organ weights of treated animals with respective control animals on day 29 were found to be comparable with respective control group. Gross pathological examination of animals did not reveal any abnormalities. Histopathology: The vital organs such as liver, heart, Spleen and kidneys were removed from the test groups at the end of the study macroscopically did not reveal any abnormal macroscopic changes. Microscopically, these organs of the test groups revealed normal histological appearance when compared with the control group (Panel 1-4).

CONCLUSION

The median lethal dose for *Mandoora chooranam* above 2000 mg/kg body weight, and results of 28 days repeated oral toxicity study revealed there is no significant changes found at 400 mg/kg body weight. So, it can be concluded that the *Mandoora chooranam* can be prescribed for therapeutic use in human with the dosage recommendations of upto 400mg/kg body weight p.o.

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