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ACUTE AND 28 DAY REPEATED ORAL TOXICITY STUDY OF SIDDHA HERBOMINERAL FORMULATION LINGA MATHIRAI IN RATS

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ABSTRACT

The Herbo mineral formulation Linga mathirai is indicated for Diabetes mellitus in Siddha system. The drug Linga mathirai is made as per Siddha literature. Recent scientific trends insisting that administration of metals in human usage should undergo the toxicity studies. The aim of the present investigation was to evaluate acute and sub acute toxicity of the Linga mathirai on wistar rats following OECD guidelines. In acute oral toxicity study, a single dose of Linga mathirai was administered and observed for 14 days, no abnormal signs upto the dose level of 2000mg/kg body weight. Sub-acute toxicity studies were carried in two different groups of Linga mathirai was administrated orally to rats once daily for 28 days in various doses ranging from 100 and 200 mg/kg for rat respectively. The study concludes there were no change in behaviour movements and no characteristic clinical sign of toxicity or mortality observed.

KEYWORDS: Linga Mathirai, Acute toxicity, Repeated 28 days toxicity, OECD guidelines.

INTRODUCTION

Siddha, a traditional method of treatment which is primarily followed, even now, in the southern part of Indian, specifically in the state called Tamil Nadu. Siddha system of medicine was introduced by Siddhar; they were believed to be the greatest scientists in ancient times. Their works were written in Tamil poetry form in palm manuscripts. The plants, animal, metal and mineral kingdom were used as a source for preparing the medicine. Mineral source of the medicine has more potent than any other sources. Linga mathirai is one

among those medicines made as Herbo mineral formulations. The Drug Linga mathirai is indicated for Diabetes mellitus.

The issues related to lack of scientific evidence about the efficacy and safety of herbo mineral remedies remains unresolved.[1] A preclinical toxicity study is mandatory in determining a safety dose for human trial.[2] Hence, the traditionally practised medicine has to be validated by the modern techniques to prove the safety and efficacy of the drug. So, in this study, an effort was made to evaluate toxicity of the Herbo mineral formulation Linga mathirai.

MATERIALS AND METHODS

Identification and authentication

All the raw drugs were identified and authenticated by Botanist and experts of Gunapadam Department (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai.

Preparation of Linga mathirai

Aconitum ferox (Naabi) has grounded well with water and made as paste, which was used to seal the cinnabar (Lingam). Then the root of *Indigofera aspalathoides* (Sivanar vembu) is grounded with water made as paste and seal the above. The seal was covered by silk cloth kept it in sunlight for two hours. Then the gingelly oil was poured in iron pan, the above sealed one was fried until the gingelly oil has evaporates completely. Then the seals are removed, the cinnabar was grounded with boiled rice gruel for three hours and rolled it as pills.[3]

Animal

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under CPCSEA (Approval no: IAEC/XLIV/26/CLBMCP/2014) at C.L. BaidMetha College of pharmacy, Thuraipakkam, Chennai.

Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house of king's institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within ±20% of the mean weight of the animals. The studies were conducted in the animal house of C.L. Baid Metha College of pharmacy, Thuraipakkam, Chennai.

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute oral toxicity – OECD Guidelines – 423

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co – operation and Development) Guideline-423.

Linga Mathirai was prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavages using a feeding needle. Animals were fasted 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, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours 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.

Repeated 28 days oral toxicity study – (OECD-407 guidelines)

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *Linga Mathirai* was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for

the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route was considered to be a proposed therapeutic route.[4]

Preparation and administration of dose

Linga Mathirai at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

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's multi comparision test using a computer software programme GRAPH PAD INSTAT-3 version.

RESULTS

Acute oral toxicity in rats– OECD 423

Wistar albino rat was treated with the test drug *Linga Mathirai* of single dose of 2000mg/kg in 2%CMC as suspension. This study was conducted as per the OECD guidelines. The result of acute toxicity of *Linga Mathirai* has been tabulated below.

Table 1: Observation in acute toxicity study.

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle grippness	Normal
Rearing	Mild
Urination	Normal

Table 2: Dose finding experiment and its behavioural Signs of Toxicity for *Linga Mathirai.*

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 9Convulsions 10.Muscle Spasm 11.Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18.Writhing 19 Respiration 20.Mortality

Results of 28-days repeated dose study in rats

Wistar albino rat was treated with the test drug *Linga Mathirai* for 28 days repeated dose of 100mg/kg and 200 mg/kg in 2% CMC as suspension. This study was conducted as per the OECD guidelines. The result of sub acute toxicity of *Linga Mathirai* has been tabulated below.

Body Weight

Table 3:- Effect of *Linga Mathirai* on Body Weight of rats.

Dose (mg/kg/day)	Days				
	0	7	14	21	28
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
100	126.02±1.15	129.86±1.57	132.94±1.51	135.77±0.76	136.78±1.14
200	127.49±1.24	130.92±0.89	133.83±1.53	135.98±0.92	138.81±0.81

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant,*

***P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Organ weight

Table 4: Effect of *Linga Mathirai* on Organ weight of rats.

Organ	Control	100 mg/kg	200 mg/kg
Liver (g)	5.24±0.14	5.1±0.24	5.2±0.3
Heart (g)	0.70±0.05	0.65±0.12	0.72±0.11
Lung (g)	1.78±0.25	1.66±0.42	1.73±0.4
Spleen (g)	0.74±0.07	0.68±0.17	0.78±0.08
Brain (g)	1.43±0.18	1.2±0.41	1.45±0.36
Kidney (g)	0.70±0.05	0.61±0.11	0.69±0.09

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Haematological parameters

Table 5: Effect of *Linga Mathirai* on Haematological parameter of rats.

Parameter	Control	100mg/kg	200 mg/kg
RBC(x 10^6/mm^3)	8.29±0.43	9.25±0.46	9.61±0.40
PCV (%)	49.66±0.77	50.92±0.73	52.22±1.06
Hb (%)	15.13±0.39	15.69±0.37	15.86±0.24
WBC(x 10^3/mm^3)	11.75±0.85	10.35±0.34	9.46±0.34
Neutrophils (%)	23.29±0.73	26.91±1.24	28.13±1.14
Eosinophills (%)	4.1±0.23	3.43±0.52	2.70±0.64
Lymphocyte (%)	85.5±0.46	85.78±0.21	86.5±0.41
Platelets(x 10^3/mm^3)	425.73±1.35	427.02±0.98	424.54±2.19

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant*

Biochemical parameters

Table 6: Effect of *Linga Mathirai* on Biochemical parameter in rats.

Parameters	Control	100 mg/kg	200 mg/kg
Glucose (mg/dl)	108.63±0.81	107.14±0.56	105.95±0.78
BUN (mg/dl)	22.06±1.55	24.45±1.86	25.70±2.39
Creatinine (mg/dl)	0.85±0.07	0.83±0.06	0.76±0.03
SGOT (U/L)	74.35±1.23	72.93±1.15	71.43±1.24
SGPT(U/L)	27.07±0.84	25.49±1.17	23.94±0.97
ALP (U/L)	104.63±1.14	103.43±1.69	102.71±0.71
Protein (g/dl)	8.58±0.68	8.24±0.53	7.66±0.43
Albumin (g/dl)	5.34±0.40	4.77±0.31	4.11±0.14
Total Cholesterol (mg/dl)	93.21±1.16	92.01±0.64	90.02±1.02
Triglycerides (mg/dl)	52.58±1.56	55.99±1.46	64.23±1.59

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Urine Parameter

Table 7: Effect of *LingaMathirai* on Urine parameter in rats.

Parameter	Control	100 mg/kg	200 mg/kg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Turbid
Specific gravity	1.01	1.02	1.04
pH	7.2	7.4	6.9
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketone	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelialcells	Nil	Nil	Nil
Casts	Nil	Nil	Nil

DISCUSSON

All the animals from control and all the treated dose groups up to 200 mg/kg survived throughout the dosing period of 28 days. The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall mild weight gain throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal. The weights of organs recorded did not show any significant differences in the treatment and the control group indicating that *Linga Mathirai* was not toxic to kidney, liver and spleen. Hematological analysis conducted at the end of the dosing period on day 29, revealed no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical analysis conducted at the end of the dosing period on day 29, No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits. Urine analysis data of control group and the test groups of animals taken on 28^{th} day showed no abnormal results.

CONCLUSION

Based on these above findings, there was no toxic effect observed up to 200 mg/kg of Linga mathirai treated over a period of 28 days. Hence, it can be concluded that the Linga mathirai

can be prescribed for therapeutic use in human with the dosage recommendations of up to 200 mg/kg body weight p.o.

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