Research article

Studies on the safety profiles of a *Siddha* preparation -*Thirithodamathirai*

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ABSTRACT

Introduction and Aim: *Thirithodamathirai* (TTM)is aherbo metallic formulation of *Siddha* system of medicine also called as *Veeramathirai* or *Mukkutra mathirai*. Therapeutically it is recommended for all types of fever, delirium, and lung infections. It has only two ingredients namely *veeram*(Mercuric chloride,20%)and pepper seeds (80%). In this study, *Veeram* was detoxified and the test sample *Thirithodamathirai* prepared as per the instruction given in the classical siddha text *Siddha vaithiyathirattu*. It is a highly poisonous substance and if prepared improperly will have toxic effects.

Materials and Methods: Plant materials used to detoxify the toxic effect of *veeram* are bitter gourd, milk, lemon juice and tender coconut. In the present study, the method using lemon juice was used in the detoxification procedure. After detoxification, *veeram*was used in preparing the *mathirai*. This research work focuses on studying the safety profiles of *Thirithoda mathirai* by carrying out *in vivo* single dose toxicity studies in female Wistar rats. *Thirithoda mathirai* was dissolved in purified water, was administered to the experimental animals at a dose level of 50, 300 and 2000 mg/kg body weight and were observed for 14 days.

Results: Single dose oral administration of the *mathirai* at a dose of 50 mg/kg caused no harmful toxic effects on the body mass / body weight changes and food intake in *Thirithodamathirai* treated female rats. Dose levels of 300 and 2000 mg/kg showed 33.3 and 100% mortality respectively.

Conclusion: The acute oral LD_{50} of *Thirithodamathirai* for Wistar rats was observed to be 500mg/kg body weight.

Keywords: *Thirithodamathirai;* mercuric chloride; pepper seeds; acute toxicity.

INTRODUCTION

In the *Siddha* system, medicines are classified into two types: Medicines for internal use and external use. Internal medicines are classified into 32 categories like decoctions (*Kudineer*), juice (*saaru*), powder (*choornam*), paste (*karkam*) etc. *Mathirai* is one of the internal medicines that refers to pills and is also called as *kuligai* or *urundai* (1). It is prepared from finely ground paste of drug rolled into pills or pressed into tablet (2). *Mathirai* will retain its potency for one year (1). For the present work, *Thirithoda mathirai* (TTM), one of the *Siddha* herbo-metallics was selected. It is also called as *Veera mathirai* or *Mukkutra mathirai* (3).Therapeutically it is used for all types of fever like enteric fever, delirium, and lungs infections. It has only two ingredients: Mercuric chloride and pepper seeds (4). In *Siddha* medical system, mercuric chloride (Hg_2Cl_2) is called as *veeram* (5). Clinically *veeram* based medicines are used to cure osteoarthritis, generalized body pain, sexually transmitted disease like syphilis or gonorrhea, stomach ulcer, tumor,and carcinoma (3). The toxicology studies and significance of this medicine has been well documented in detail (6).

Veeram is a highly poisonous substance. The toxic effects of *veeram* in case of improper preparations have also been prescribed in *Siddha* textbooks (7). In *Siddha* system *veeram* is used in combination with other drugs after proper processing and detoxification. Usually, plant materials such as bitter guard, milk, lemon juice and tender coconut are used in detoxifying the *veeram* (8). In the present study, lemon juice method was used for detoxification. After detoxification, *veeram* was used for preparing TTM. In the year 2012 Sathish *et.al* explained the preparation protocols involved in the standardization of the *Veeram* based medicine *Mega sanjeevi mathirai* (9). *Thirisootha mezhugu*, another *Veeram* based Siddha medicine was detoxified using lemon juice method (10). In this process one earthen pot filled with lemon juice (1250 ml) and sample (50 gm) was taken and tied with a kada cloth tightly. Then the *thulayanthiram* method was used to bundle and boil the sample with limestone water (3,11). Another ingredient is black pepper seed, botanically equated as *Piper nigrum* L. It is an important and essential herbal

drug in all traditional health care systems. In *Siddha*, it acts as an antidote, carminative, antiperiodic, stimulant and *antivatha* drug. Therapeutically it is used in the management of cold, cough, fever, wheezing, sinusitis, indigestion, itching and insect bites (12). Pepper and processed *veeram* were ground with pepper decoction. Decoctions are the simplest, effective, easily digestible, and fast curable natural medicine form. It is one of the thirty-two types of internal medicine (3). In an earlier report by Sangeetha *et al*. the *veeram* based formulation *Ayaveera centhuram* was studied from toxicological point of view using Swiss albino mice (13). In another study, toxicological profiles of *Gowrichinthamani centhuram* was reported (14).

The aim of this study is proven the safety profiles of *Thirithoda mathirai* by carrying out *in vivo* single dose toxicity studies on female Wistar rats. This research work has three steps: 1. Detoxification of *veeram* by lemon juice method mentioned in the *Siddha* literature *Sarakku suthi sei muraikal*; 2. Preparation of TTM as per the *Siddha* literature *Siddha vaithiya thirattu* using purified *veeram*; 3. Acute oral toxicity (AOT) test on female Wistar rats (*Rattus norvegicus*).

MATERIALS AND METHODS

TTM Preparation

Veeram was procured from the raw drug dealers of Chennai market and authenticated using the database (7487-94-7 CAS Data Base). Before use, the *veeram* was detoxified and then taken for preparing TTM. TTM was prepared from purified *veeram* and pepper seed ground with pepper decoction for twelve hours and this paste was rolled into 100 mg pills (2). Fig.1 explains the preparation method of TTM. It is advisable to serve the medicine only after a month of its preparation. Dosage is 1-2 pills with black pepper decoction.

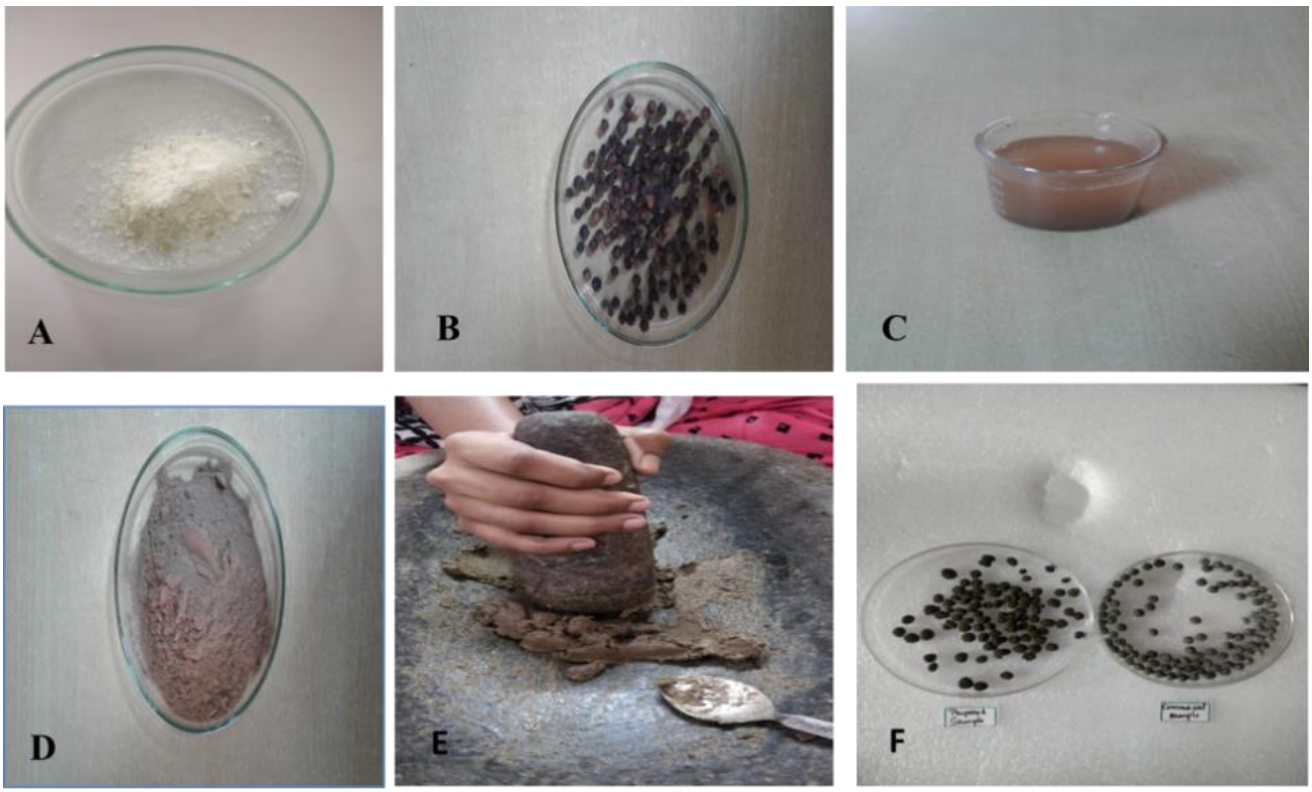

Fig.1: Preparation of TTM.A: Raw *veeram*; B: Pepper; C: Pepper decoction. D: Raw *veeram* and pepper powder; E: TTM grinding; F: TTM- in house preparation (commercial sample)

Toxicity study design

Acute oral toxicity studies were carried out using three Wistar rats of unisex in one stage. Dependent upon the death rate and/or the moribund status of the rats, on regular 2-4 stages may be essential to allow conclusion on the acute toxicity. The study was conducted[15] as per ethical committee guidelines 423. The study was performed after obtaining the necessary approval by the Institutional Animal Ethical Committee, SASTRA Deemed University (IAEC Approval Number: 335/SASTRA/IAEC/RPP).

Experimental animals

In this study female Wistar rats were used. The animals were 8 to 12 weeks old, nulliparous, and non-pregnant. Rats were fed with commercial pellet purchased from regular rodent pellet feed supplied by M/s. ATNT Laboratories, Mumbai, India, and RO water *ad libitum* and were acclimatized for seven days before the conduct of the study.

Animal house condition

All the investigational animals were preserved under usual conditions (Temperature: 22±3°C and relative humidity: 50 to 70%) throughout the study time. The investigational room was kept the condition with a12h brightness and 12h shadowy lighting condition using automatic timer. Ordinary polypropylene mice cage with stainless steel top grills were utilized to house the animals. The cages were autoclaved. Sieved and sterilized paddy husk was used as the bedding material. Animals were housed individually. Bedding material, cages, grills, and water bottles were changed in three days once.

Preparation of TTM drug and animals

TTM was suspended in purified water, given to all the rats at a dose level of 50, 300 and 2000 mg/kg body mass and were noted for 14 days. All the animals were fasted during the night before drug treatment. Separated the animals into two groups, with every group having three animals for 50 mg and 300 mg dosages respectively. The TTM was given by oral gavages using squeeze and stainless-steel ball - angled oral gavage needle. Throughout the examination period, all the rats were observed two times a day for mortality. Body mass of every animal was recorded just before to the TTM treatment (Day 0), Day 7 and 14 using an electronic animal weighing scale (Sartorius AG, Germany). The feed intake of each animal was noted every day in the whole study period.

Observation of toxicity signs

All animals were examined for medical symptoms of toxicity like changes in dermal, hair growth, visual system and inner lining of mouth, nose and body cavities, occurrence of discharge and excretions and autonomic activity, changes in walking position and response to handling, presence of colonic or tonic movements, stereo types, and bizarre behavior during the examination period. Clinical signs including death, suffering from a disease or medical condition, commonly look the dermal, hair growth, visual system and inner lining of mouth, nose and body cavities, nervous system etc and performance were noted. The clinical remarks such as excretion and reliability, wet yellow urogenital staining, dried yellow urogenital staining, clean wet matting around mouth, clear ocular discharge, hyper activeness, skin and fur examination, tremor and convulsion, piloerection, change in gait, repetitive circling and excessive grooming were also monitored in all test drug treated experimental animals.

RESULTS

According to the *veeram* detoxification data, it was observed that lemon juice treated samples showed absence of heavy metals and increased purity of mercury as compared with other purification methods

Single dose toxicity study

In this study, no death was noted in the dose level of 50 mg/kg b. wt. treated group of rats throughout the examination period, whereas rats treated with TTM at the dose levels of 300 and 2000 mg/kg showed 33.3 and 100% mortality respectively (Table 1). Similar results were obtained during the acute toxicity study on the mercury-based Siddha formulation *Arumuga chenthuram* by Murugan *et al*., (16).

Table 1: Mortality data of experimental animals

Group	Dose (mg/kg body weight)	Percentage mortality (upto 14 days)
TTM treated (3 female rats in each group)	50	0
	300	33.3
	2000	100

Body weight

The animals treated orally with TTM at the dose level of 50 mg/kg showed significant change in body weight gain on day 7 and 14 when compared with Day 0. The weekly body weight of the rats treated with 300 mg/kg of TTM showed increase in body weight but was not significant (Tables 2 to 4). The weekly body weight on Day 0 of the rats administered with 2000mg/kg is given in Table 5. The rats did not survive beyond Day 0 at a dose of 2000 mg/kg.

Feed intake

Every day food taking of the test animals with 50 mg/kg of TTM remained unaffected whole experimental phase of 14 days, whereas rats treated with TTM (300 mg/kg) showed significant decrease in feed intake up to 7 days after TTM treatment (Table 5 and 6).

Table 2: Weekly bodyweight observed in female

Animal ID	Dose (mg/kg body weight)	Sex	Body Weight (g)		
			Day 0	Day 7	Day 14
6732	50	Female	174.56	192.80	198.79
6733			174.80	191.97	199.70
6734			178.98	193.93	204.77
6774			175.40	203.46	212.91
6775			172.15	190.75	205.86
6776			174.94	191.89	204.61
Mean			175.13	194.13	204.44
SD			2.20	4.68	5.06

Table 3: Weekly body weight observed in female rats treated orally with 300 mg/kg body weight

Animal ID	Dose (mg/kg bwt)	Sex	Body Weight (g)		
			Day 0	Day 7	Day 14
6786	300	Female	167.99	190.40	195.41
6787			169.63	D	D
6788			176.69	170.11	174.93
6891			166.52	150.23	178.80
6892			177.81	D	D
6893			182.97	204.21	206.75
Mean			173.60	178.73	188.97
SD			6.51	-	-

D- Death

Table 4: Weekly body weight observed in female rats treated orally with 2000 mg/kg body weight

Animal ID	Dose (mg/kg b.wt)	Sex	Body Weight (g)		
			Day 0	Day 7	Day 14
7009	2000	Female	198.51	D	D
7010			190.78	D	D
7011			186.59	D	D
Mean			191.96	-	-
SD			6.05	-	-

D- Death

Table 5: Daily feed intake (g) by rats treated orally with TTM at the dose level of 50 mg/kg. b wt.

Animal ID	Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
6732	20.09	21.86	16.21	20.85	19.64	16.41	18.56	16.82	15.68	16.83	18.23	17.53	15.84	16.73
6733	20.1	19.66	17.47	19.46	18.67	16.61	18.82	18.85	18.94	16.95	17.65	15.36	18.6	16.97
6734	20.72	18.52	18.13	18.33	17.1	15.64	19.86	18.51	15.72	19.61	17.82	18.27	17.11	18.89
6774	21.22	24.18	21.31	21.94	18.59	22.59	20.67	22.5	21.88	20.68	20.49	23.45	20.53	20.96
6775	20.87	22.18	20.54	19.74	20.18	20.98	17.74	19.38	21.24	17.98	21.28	19.8	20.02	20.02
6776	18.37	17.87	16.2	14.92	15.57	16.35	17.3	16.83	16.67	18.59	16.68	14.65	17.5	19.34
Mean	20.23	20.71	18.31	19.21	18.29	18.10	18.83	18.82	18.36	18.44	18.69	18.18	18.27	18.82
SD	1.01	2.43	2.17	2.44	1.70	2.92	1.27	2.09	2.76	1.51	1.79	3.20	1.80	1.68

SD = Standard deviation

Table 6: Daily feed intake (g) by rats treated orally with TTM at the dose level of 300 mg/kg. b wt.

Animal ID	Day													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
6786	11.58	12.7	14.94	17.8	15.54	16.72	17.73	17.52	19.02	19.37	20.04	19.11	16.9	19.06
6787	6.67	4.31	D	D	D	D	D	D	D	D	D	D	D	D
6788	5.24	4.02	3.43	4.05	4.78	9.46	9.53	12.38	17.08	16.68	18.76	18.25	20.51	19.24
6891	7.59	6.33	9.81	7.86	7.49	10.46	9.7	12.03	12.97	15.51	15.28	16.6	17.37	14.89
6892	10.27	1.82	0.06	0.03	D	D	D	D	D	D	D	D	D	D
6893	13.56	11.49	16.64	17.06	18.07	16.96	21.23	19.57	18.93	19.5	19.35	20	18.79	18.74
Mean	9.15	6.78	8.98	9.36	11.47	13.40	14.55	15.38	17.00	17.77	18.36	18.49	18.39	17.98
SD	3.18	4.38	7.16	7.87	6.34	3.99	5.87	3.76	2.83	1.99	2.12	1.45	1.62	2.07

SD= Standard deviation;

Toxicity signs

No visible toxicity signs were observed in treated rats at the dose level of 50 mg/kg. Visible signs of toxicity such as piloerection, tremor, nasal discharge, change in gait and respiratory distress were noted in rats treated with TTM at the dose level of 300 mg/kg during the entire period of observation. Rats treated with test substance at the dose level of 2000 mg/kg died within 6 hours of TTM treatment (Table 7).

Table 7: Toxicity signs observed in female rats

Sl. No.	TS ID	Sex	Animal ID	Dose mg/kg	Day of observation	Cage side individual clinical Observation
1	TTM	Female	6732	50	0 to 14	Normal
2			6733	50	0 to 14	Normal
3			6734	50	0 to 14	Normal
4			6774	50	0 to 14	Normal
5			6775	50	0 to 14	Normal
6			6776	50	0 to 14	Normal
7			6786	300	0 to 1	Piloerection
					2 to 5	Dullness
					6 to 14	Normal
8			6787	300	0 to 2	Dullness
					0 and 2	Piloerection
					2	Piloerection, dullness, Tremor, Moribund
9			6788	300	0and 1	Piloerection
					2 to 10	Dullness
					11 to 14	Normal
10			6891	300	0 and 1	Piloerection
					2 to 14	Normal
11			6892	300	0 and 1	Piloerection
					2 to 4	Dullness
					2 and 3	Change in gait
					4	Piloerection, nasal discharge, respiratory distress, Found dead
12			6893	300	0 and 1	Piloerection
					2 to14	Dullness
13			7009	2000	0	Death within 6 hours
14			7010	2000	0	Death within 6 hours
15			7011	2000	0	Death within 6 hours

Table 8: Gross pathology of experimental animals

S. No.	TS ID	Sex	Animal ID	Dose mg/kg	Gross observations
1	TTM	Female	6732	50	NAD
2			6733	50	NAD
3			6734	50	NAD
4			6774	50	NAD
5			6775	50	NAD
6			6776	50	NAD
7			6786	300	NAD
8			6787	300	NAD
9			6788	300	External: NAD Internal: Kidneys: Pale, diffuse, marked, bilateral. A small capsulated mass found in the momentum between liver and stomach
10			6891	300	External: NAD Internal: Kidneys: Pale, diffuse, marked, bilateral.
11			6892	300	Dead
12			6893	300	NAD
13			7009	2000	Dead
14			7010	2000	Dead
15			7011	2000	Dead

Gross pathology

In the single dose treatment, the animals did not produce any sign of toxicity and no death was occurred up to a dose of 50 mg. One rat died at the dose level of 300 mg out of six animals while100% mortality was observed at 2000 mg. The kidney of the TTM treated animals showed normal features at 50 mg. However, the gross pathology of animals treated with300mg appeared pale, diffuse, marked and bilateral. The gross pathology of rats treated with TTM is summarized in Table 8.

DISCUSSION

The data of this research work showed that Single dose oral administration of TTM at 50 mg/kg caused no adverse toxic effects on the body weight and feed intake in TTM treated female rats. However, single dose oral administration of TTM at 300 mg/kg caused adverse changes in feed intake, body weight and toxicity signs such as piloerection, tremor, nasal discharge, change in gait and respiratory distress were observed. Two rats died during the first week of observation period. The acute oral LD_{50} of TTM in Wistar rats was observed to be 500mg/kg body weight. This is in accordance with a similar acute toxicity study on a mercury-based *Siddha* formulation *Linga centhuram* carried out previously in which the LD_{50} was also found to be 500mg/kg body weight (17). Depending upon this study data we conclude that the therapeutic dose level of 100 mg is very safe for human consumption.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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