

ORIGINAL RESEARCH ARTICLE

Acute and Sub Acute Toxicity Study on Siddha Drug *Thennangal Thiravagam*V Aruna¹, P Parthiban¹, V Thanigaivelan²¹Maruthuvam Branch, Government Siddha Medical College, Chennai, Tamil Nadu, India,²Sairam Advanced Centre for Research, Chennai, Tamil Nadu, India

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ABSTRACT

The herbo-mineral formulation *Thennangal thiravagam* (TT) has been used for the treatment of Bronchial asthma (BA). BA is one of the major respiratory disorders in clinical practice. As a mandate, steps were taken to evaluate safety profile of TT in mice using OECD guidelines. In Acute oral toxicity study, a single dose of TT was administered and observed for 14 days. The results of acute toxicity study of TT revealed no mortality, abnormal signs and behavioral changes in mice at the dose of 2.4ml/ kg body weight. Sub-acute toxicity studies were carried in four different groups in which TT was administered orally to mice once daily for 28 days in various doses ranging from 65, 325 and 650 µl/kg body weight respectively. Detailed hematological, biochemical, necropsy and histo-pathological evaluation of organs were performed for all animals. TT was well tolerated and no toxic manifestations were seen in any animal. Histopathological analysis revealed that Spleen, Testes, Pancreas, Lung, Intestine, Stomach, Liver, Brain, Heart, Ovary, Uterus and Kidney tissues of treated groups did not show any signs of toxicity. So TT was found to be safe in animals and no toxic effect was observed upto 650 µl/kg in both acute and sub-acute toxicity studies.

Key words: Thennangal thiravagam, Bronchial asthma, acute toxicity, sub-acute toxicity.

INTRODUCTION

Swasa kasam (Branchial Asthma) is a disease characterized by hyper-reactive airways, leading to episodic, reversible broncho constriction, owing to increased hyper-responsiveness of the trachea-bronchial tree to various stimuli^[1]. Urbanization, exposure to tobacco smoke, chemical irritants appears to be important risk factors in the incidence of asthma^[2]. The occurrence of asthma has increased significantly since the 1970s. In 2011, 235-300 million people have been diagnosed with asthma and approximately 2, 50,000 - 3, 45,000 people die every year from the disease. Globally, Bronchial Asthma causes moderate or severe disability in 19.4 million people as of 2004 (16 million of which are in low and middle income countries). Worldwide, the economic costs associated with asthma are estimated to exceed those of TB/ AIDS combined^[2].

There are many alternative treatments available that can treat the BA. Herbs and minerals have been in use since long time to treat various diseases^[3]. However, many issues related to a

lack of scientific evidence about the efficacy and safeties of the drugs remain unresolved^[4,5]. The Pre-clinical toxicity studies were essential for determining a safe dose for human trials^[6].

The interventional Siddha drug *Thennangal thiravagam* (TT) quoted in the siddha literature Yakobu vaithiya chinthamani has been used for the treatment of Swasa kasam (Bronchial Asthma)^[7]. Consequently an effort was made to evaluate acute and sub-acute toxicity of the herbo-mineral siddha formulation TT in laboratory animals.

MATERIALS & METHODS**Aim:**

Aim of the study is to evaluate the acute and sub-acute toxicity of the siddha drug '*Thennangal thiravagam*'.

Preparation of the *Thennangal thiravagam*:

a) **Ingredients:** Purified Thalagam (Arsenic trisulphide), Thennangal (Coconut toddy).

b) Procedure:

Thalagam is purified using pannangal (palm toddy) and made into powder form. Coconut toddy is fermented for 6 days and using

Distillation apparatus, Thiravagam is taken. Then purified Thalagam is mixed with Thiravagam and once again using the same apparatus Thiravagam is taken.

Animals:

BALB/C Mice of either sex weighing more than 20gms 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/1-10/2013). All the animals were kept under standard environmental condition ($23\pm 2^{\circ}\text{C}$), standard light cycle (12 h light, 12 h dark). The animals had free access to water and standard pellet diet. The animals were 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^[8,9]

Acute oral toxicity study for *Thennangal thiravagam* was carried out as per OECD Guidelines 423. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance was administered in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing and weighed before the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours.

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 behaviour 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 behaviour pattern. The animals were then observed daily for gross behavioural changes and any other signs of acute toxicity (**Table 1**).

Sub-Acute Toxicity –OECD 407 Guidelines¹⁰:

In a 28-days, sub-acute toxicity study, ten mice (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 *Thennangal thiravagam* (p.o.) for 28 days at a dose of $65\mu\text{l/kg}$ (x), $325\mu\text{l/kg}$ (5x) and $650\mu\text{l/kg}$ (10x) body weight respectively.

The weight of each mice was recorded on day 0 and weekly throughout the course of the study (**Table 2**).

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 in mice

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

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

Sub-acute oral toxicity 28-day repeated dose study in mice

Table 2: Body weight (g) changes of mice exposed to Thennangal thiravagam

Dose ($\mu\text{l/kg/day}$)	Days				
	1	7	14	21	28
Control	22.37 \pm 3.21	24.14 \pm 4.09	28.21 \pm 2.17	27.21 \pm 5.11	33.32 \pm 1.89
65	24.16 \pm 1.42	26.92 \pm 1.67	33.85 \pm 1.32	34.25 \pm 1.68	35.68 \pm 0.58

325	23.14±1.1	24.8±1.58	27.2±0.89	28.9±1.48	32.8±0.72
650	22.47±1.16	24.5±0.46	29.8±0.78	34.5±1.36	36.8±0.69

Values are expressed as mean ± S.E.M. N=10

Hematological analyses: At the end of the study, all animals were kept fasted for 18 h and then anaesthetized with anaesthetic 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 3**) (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semi-automated hematology analyzer.

Table 3: Effect of Thennangal thiravagam on Haematological parameters in mice

Parameter	Control	65 µl/kg	325 µl/kg	650 µl/kg
RBC (x 10 ⁹ /mm ³)	5.2±0.43	5.4±0.9	5.32±0.46	5.16±0.3
PCV (%)	48.2±1.8	49.6±0.4	51.2±1.2	48.3±0.9
Hb (g/dl)	15±0.3	15.2±0.6	15.8±0.8	14.8±0.9
WBC (mm ³)	8422±183	8634±111	8780±134	9247±153**
Neutrophils (%)	18±2	26±2.2**	24±1.3*	24±0.89*
Mononuclear cells (%)	78±3	72±1.56	75±0.78	73±2.54
Eosinophils (%)	3±0.3	2.2±0.15**	1.8±0.06**	1.4±0.07**
Platelets (x 10 ³ /mm ³)	645±6.2	631±9.2	626±1.5	652±4.3

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=10

Biochemical parameters: Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental mice were analyzed for protein, bilirubin, urea, creatinine, triglyceride, cholesterol and glucose levels 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 4**).

Table 4: Effect of Thennangal thiravagam on biochemical parameters in mice

Parameters	Control	65 µl/kg	325 µl/kg	650 µl/kg
Protein (g/dl)	6.33±0.3	6.2±0.5	5.9±0.28	5.6±0.15
Albumin (g/dl)	2.8±0.2	2.5±0.16	3.1±0.29	2.4±0.18
BUN (mg/dl)	21.33±4.6	22.4±2.8	21.6±1.5	19.8±0.98
Urea (mg/dl)	63±4.3	58.4±3.4	47.6±1.6	43.4±2.4
Creatinine (mg/dl)	0.53±0.03	0.48±0.04	0.56±0.11	0.52±0.06
Total Cholesterol (mg/dl)	111.53±13.17	98.6±11.8	99.2±5.96	113.6±8.6
Triglycerides (mg/dl)	97.56±14.5	96.4±11.2	116.4±9.8	120.6±6.8
Glucose (mg/dl)	113.4±12.2	99.4±6.8	116.4±11.3	120.6±9.5
Total Bilirubin (mg/dl)	0.9±0.08	0.84±0.06	0.73±0.02	0.62±0.09
SGOT (U/L)	86.5±5.0	88.4±9.8	91.3±6.5	96.2±5.8
SGPT (U/L)	46.5±6.2	43.6±5.6	45.4±2.9	51.2±3.2
Alkaline phosphatase (U/L)	48.6±7.2	46.4±5.2	52.3±3.4	58.6±4.6
Sodium (mEq/L)	147.3±5.8	138.6±6.9	141.4±9.2	139.3±5.6
Potassium (mEq/L)	5.3±0.4	5.6±0.09	4.9±0.6	5.2±0.13

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; 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 5**). Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 2.4ml/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-eosin and were examined microscopically (**Panel 1, 2, 3 & 4**).

Table 5: Effect of Thennangal thiravagam on Organ weight in mice

Organ	Control	65 µl/kg	325 µl/kg	650 µl/kg
Liver (mg)	2115.9±210.5	2132.6±113.2	2364.8±98.6	2162.7±56.3
Heart (mg)	139.8±15.4	132.4±11.4	135.2±16.3	129.6±9.5
Lung (mg)	175.2±16.6	169.3±11.6	177.6±15.6	181.4±13.8
Spleen (mg)	145.5±19.3	138.2±9.6	146.4±9.4	145.3±11.6
Brain (mg)	455.6±14.2	462.3±20.3	485.4±16.5	456.7±28.6
Kidney (mg)	632.7±84.2	646.4±26.3	710.5±58.3	662.3±48.9

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=10

Table 6: Effect of Thennangal thiravagam on Urine parameters in mice

Parameters	Control	65 µl/kg	325 µl/kg	650 µl/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Clear	Yellow
Specific gravity	1.01	1.01	1.01	1.02
Ph	6.4	6.2	7.1	6.8
Protein	Nil	Nil	Nil	Nil
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	- ve	- ve	- ve
Ketones	-ve	- ve	- ve	- ve
Blood	Absent	Absent	Absent	Absent
RBCs	Nil	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil

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 *Thennangal thiravagam* revealed no mortality, abnormal signs and behavioral changes in rats at the dose of 2.4ml/ kg body weight administered orally (Table 1). The median lethal dose for *Thennangal thiravagam* should be above 2.4ml/kg and it comes under unclassified. All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub acute toxicity study. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days (Table 2).

The results of haematological investigations such as Erythrocytes, Total leucocytes and Platelets count (Table 3) conducted on day 29, revealed no significant changes in the values when compared with those of respective controls except for Total leucocytes . This gave clear justification that bone marrow and spleen were not influenced by *Thennangal thiravagam*. Regarding Total leucocytes count , there is a slight increase in the *Thiravagam thiravagam* at the dosage of 65µl/kg, 325µl/kg and 650 µl/kg. The other parameters were within the normal limits. Results of Biochemical investigations conducted on days 29 and recorded in (Table 4), revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits. Triglycerides level was elevated in 325µl/kg dose group (P<0.05) and at the dosage of 650µl/kg, was slightly increased but these were within the normal limits. The other cardio vascular risk markers were also within normal ensured that *Thennangal thiravagam* did not influence the Cardio vascular system.

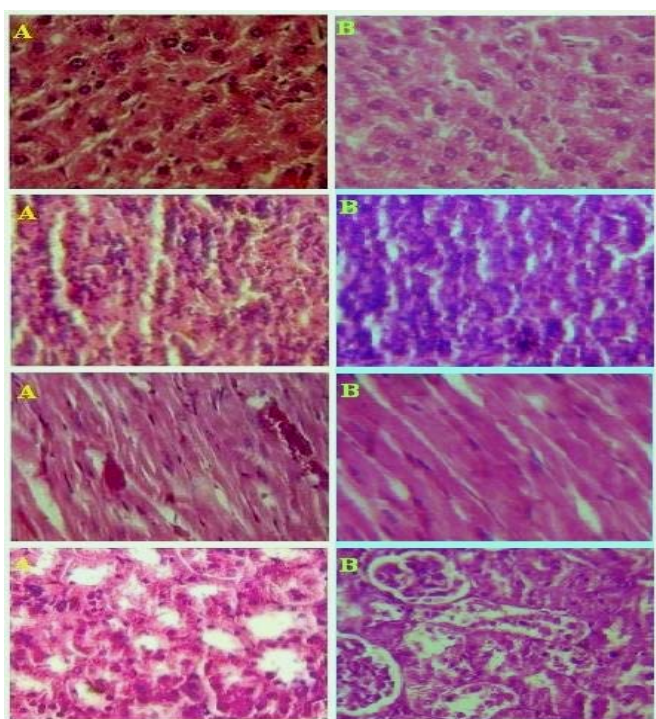
**Panel 1: Light photomicrography of liver of mice**

Figure A – Control

Figure B – Treated on high dose, no abnormality is seen in hepatocytes, sinusoids.

Panel 2: Light photomicrography of Spleen of mice

Figure A – Control

Figure B – Treated on high dose, no abnormality is seen in trabeculae, capsule.

Panel 3: Light photomicrography of Heart of mice

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 mice

Figure A – Control

Figure B – Treated on high dose, no abnormality is seen in glomeruli, Bowman's capsule, capillaries.

Urine analysis data (**Table 6**) of control group and treated group of animals determined in week 4 did not reveal no abnormalities. Group Mean Relative Organ Weights (% of body weight) are recorded in (Table 5). Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group. Gross pathological examination of animals in control as well as the treated groups 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 and carefully observed macroscopically to find any observable gross lesions compared with the control group and 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 acute and sub-acute toxicity study of *Thennangal thiravagam* revealed no toxicity by oral route over a period of 28 days. So, it can be concluded that *Thennangal thiravagam* can be prescribed for therapeutic use in human with the dosage recommendations of upto 2.4ml/kg body weight p.o.

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