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EVALUATION OF ACUTE AND SUB-ACUTE TOXICITY OF THE SIDDHA DRUG - THALAGA KULIGAI

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ABSTRACT

Aim: The aim of the study is to evaluate the acute and sub-acute toxicity of Thalaga Kuligai on Rats as per OECD Guidelines. Thalaga Kuligai is a potent herbo- mineral Siddha drug mentioned in *"Agasthiyar Vaithiya Kaviyam 1500"*. **Methodology**: Acute toxicity study was carried out using wistar Albino rats as per OECD guidelines 423.Thalaga Kuligai in 1% SCMC was administered as a single oral dose of 2000 mg/Kg and the animals were observed for any behavioural changes or mortality. For sub-acute toxicity study , 2 groups of Wistar albino rats were dministered the dose of 100 and 200 mg/kg/day for 28 days according to OECD guidelines 407 and the animals were observed for toxicity symptoms. All the animals were sacrificed on 29th day and examined for changes in organ weights and histology changes were noted. **Results**: The Present study results showed that there was no impairment in hepatic, renal, haemopoeitic functions which were observed throughout the study. No mortality was observed in acute and sub-acute toxicity studies.

KEYWORDS: Thalaga kuligai, Swasakaasam, Bronchial Asthma, Siddha, Acute and Sub-acute Toxicity.

INTRODUCTION

Medicine – The Art of Healing.

"Medicine is the science or practices of the diagnosis treatment and prevention of disease. It restores health by the prevention and treatment of illness".[1]

Siddha system of medicine ensures prevention and promotion of well-being through principles of "*food is Medicine and Medicine is food*" and principles involving life style modifications which enlightens its uniqueness in preventing and treating diseases. Siddha medicines are based on resources derived from herbs, metals, minerals and animal products, also weaving in itself the great art of varmam, yogam, thokkanam and many other non-medication therapies.

Asthma is an inflammatory disease of the small airways, characterized by episodic, reversible bronchial obstruction due to hyper-responsiveness of tracheo-bronchial tree to a multiplicity of intrinsic and extrinsic stimuli manifested clinically by paroxysms of polyphonic wheeze, dyspnoea, and cough which may be relieved spontaneously or as a result of therapy.[2] Bronchial Asthma which could be correlated to Swasakasam in Siddha pathology is one of the most common respiratory disorder in developed and developing countries like India. The prevalence of asthma increased steadily over the latter part of the last century, first in the developed and then in the developing world. Current estimates suggest that asthma affects 300 million people worldwide, with a predicted additional 100 million people affected by 2025.[3] In urban areas, the prevalence of bronchial asthma is increasing due to increase in environmental smoke and air pollution resulting from urbanization. It was found that the prevalence was significantly more among those with a family history of Asthma, having smoking habits in any of family members and absence of smoke outlet in the house. WHO recognizes Asthma as a disease of major public health problem[4] and plays a unique role in the co-ordination of international efforts against the disease. Asthma cannot be cured but it could be controlled.[5]

Thalaga Kuligai is a siddha herbo-mineral formulation mentioned in the Siddha literature "*Agasthiyar Vaithiya Kaviyam 1500",* indicated for *surakasam, swasakasam, Kuththirumal*. Plants and Minerals were used to treat many diseases for many years.[6] But the lack of scientific evidence about the safety and efficacy of the drugs remains unresolved.[7] Hence in this article the author has aimed to evaluate the safety of Thalaga Kuligai through the Acute and Sub- acute toxicity studies.

MATERIALS AND METHODS

Preparation of Thalaga Kuligai

The raw drugs required for the preparation of Thalaga kuligai were procured from a reputed indigenous raw drug shop in Chennai, Tamil Nadu and were authenticated at Siddha Central Research Institute, Chennai. The raw drugs were purified and the trial drug was prepared as per preparatory methods indicated in Siddha literature "*Agasthiyar Vaithiya Kaviyam 1500"*.

Animals

Rats of either sex, more than 8 weeks of age, weighing 150 to 200 gms, were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai. The animals were used with the approval of the Institute animal ethics committee (IAEC) of K.K College of pharmacy, Gerugambakkam, chennai with approval no. KKCP/2013/005/CPCSEA.

The animals were housed in Polypropylene cages provided with bedding of husk , were fed with a balanced standard pellet diet (Sai meera foods Pvt Ltd, Bangalore) and maintained under standard laboratory conditions, providing 24-28^{o}C temperature, Relative humidity between 30% and 70%, standard light cycle (12 h light, 12 h dark) and water ad libitum. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. The animals were acclimatized for one week under laboratory conditions.

ACUTE ORAL TOXICITY STUDY

Procedure

Acute oral toxicity study was carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423.[8] Healthy female rats, weighing 150–200 g, were selected and oral administration of the single dose of *Thalaga Kuligai* was done aseptically by suspending in 1% SCMC (Sodium carboxymethyl cellulose) by gavage using a feeding needle. Animals were fasted prior to dosing. They were deprived of food, but not water 12 h prior to the administration of the test substance. Following the period of fasting, the animals were weighed and then the test drug was administered.

After the drug 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 guidelines after the drug administration. An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviours of the rats were continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which the signs of toxicity or mortality occurred were recorded.[9]

The visual observations included skin changes, mobility, and aggressiveness, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then maintained for further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Justification for Dose Selection

The results of the acute toxicity studies in rats indicate that *Thalaga Kuligai* is non-toxic and no behavioural changes were observed up to a dose level of 2000mg/kg on oral administration.

REPEATED DOSE 28-DAYS SUB -ACUTE ORAL TOXICITY

Methodology

Sub-acute toxicity studies were carried out according to OECD 407[10] and ten Rats (Five Male and Five Female) in each group were randomly divided into three groups for dosing up to 28 days. Thalaga Kuligai at two doses level respectively was suspended in 1% SCMC in distilled wate*r*. It was administered to animals at the dose levels of 100 and 200 mg/kg/day.

The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection was done.[11]

Preparation and Administration of Dose

Thalaga Kuligai at two doses level respectively was suspended in 1% SCMC in distilled water. It was administered to animals at the dose levels of 100 and 200 mg/kg/day.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.

Observations

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight

Weight of each rat was recorded on day 0 and at 5 days intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

TERMINAL STUDIES

Laboratory Investigations

Following laboratory investigations were carried out on 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether 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.

Haematological Investigations

Blood samples of control and experimental rats was analyzed for haemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV).

Biochemical Investigations

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.

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. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 200 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. 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.

The organs included brain, heart, kidneys, liver and lungs of the animals were preserved they were subjected to histo-pathological examination.

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, and haematology and blood chemistry were subjected to One-way ANOVA, followed by dunnett's test using a computer software programme. (Graph Pad Prism 5.0).

RESULTS AND DISCUSSION

In the acute toxicity study, *Thalaga Kuligai* is non-toxic and no behavioural changes were observed up to a dose level of 2000mg/kg on oral administration (Table 1).

Table 1: 4 hours observation in acute toxicity studies 2000mg/kg.

Parameters observed	Ist hr	IInd hr	IIIrd hr	IVth hr
Aggressiveness	+	+	+	+
Alertness	+	+	+	+

Alopecia	-	-	-	-
Circling	-	-	-	-
Diarrhoea	-	-	-	-
Edema	-	-	-	-
Eye closure at touch	+	+	+	+
Grip strength	+	+	+	+
Grooming	+	+	+	+
Lacrimation	-	-	-	-
Loss of writing reflex	-	-	-	-
Mortality	-	-	-	-
Nasal sniffing	-	-	-	-
Piloerection	-	-	-	-
Rearing	-	-	-	-
Righting reflex	-	-	-	-
Seizures	-	-	-	-
Straub tail	-	-	-	-
Urine stains	-	-	-	-

Thalaga Kuligai was subjected to oral toxicity evaluation using a 28-day toxicity test is an accustomed practice. Sub-acute oral toxicity has been advocated as a fundamental test for assessing safety and has been applied previously in many safety assessment studies. In this report, we have first indicated that no dose-related toxicity effect was observed and in the sub-acute toxicity study there was no toxicity induced behavioural changes (Table 2) and the biochemistry parameters all fell within the reference range (Table 3).

Table 2: Behavioural changes observed during Sub-Acute toxicity period.

Parameters observed	**Day- 2**	**Day- 4**	**Day-6**	**Day-8**	**Day-10**	**Day-12**	**Day-14**	**Day-16**	**Day-18**	**Day-20**	**Day-22**	**Day-24**	**Day-26**	**Day-28**
Aggressiveness	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Alertness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pilo erection	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Biochemical Parameters.

Biochemical parameter	**Control**	***Trial drug***	
		100 mg	**200mg**
Creatinine (mg/dl)	0.5890±0.079	0.6867±0.024 ^{ns}	0.7150±0.048 ^{ns}
Urea (mg/dl)	15.30 ± 0.47	14.83±0.401 ^{ns}	14.50±0.885 ^{ns}
Triglycerides (mg/dl)	52.20±1.13	50.00±1.414^{ns}	50.83±2.120 ^{ns}
Total Cholesterol (mg/dl)	46.60±1.21	50.33±1.667 ^{ns}	46.67±2.305 ^{ns}
Total protein (mg/dl)	4.40±0.26	4.517±0.569 ^{ns}	4.483±0.526 ^{ns}
Albumin (g/dl)	3.20±0.41	4.583±0.327 ^{ns}	4.667±0.333 ^{ns}
AST (IU/L)	121.41±2.68	207.8±4.175 ^{ns}	194.8±10.691 ^{ns}
ALT (IU/L)	69.40±1.57	66.33±2.044 ^{ns}	66.83±3.497 ^{ns}
ALP (IU/L)	112.6±4.67	282.3±7.008 ^{ns}	286.7±5.264 ^{ns}
T. Bilirubin (mg/dl)	0.2569±0.32	0.3243±0.039 ^{ns}	0.3388±0.041 ^{ns}

Values are mean ±SEM from 10 animals in each group. ns no significant change When compared with untreated control group.

Body weights were increased during the period of study, there were no significant difference ($P > 0.05$) in body weights in male and female rats or between treatment and control groups (Table 4,Table 5).

Table 4: Change in body weight.

Treatment	0th day	5th day	10th day	15th day	20th day	25th day	28th day	% increase
Control	175.83±6.84	179.50±6.28	181.83±6.46	184.83±6.31	187.16±6.01	190.66±6.46	193.66±5.70	9.79
100mg/kg	177.83±6.28	179.83±6.92	181.33±6.95	184.66±6.97	187.50±7.28	189.33±7.03	192.66±6.81	8.33
200mg/kg	186.66±6.57	187.50±6.65	190.83±6.17	193.00±6.34	196.00±6.69	199.00±7.05	202.00±6.96	7.42

Values are mean ±SEM from 10 animals in each group

Table 5: Relative Organ Weight.

Dose	Relative Organ Weight of rats					
	Liver	Kidney	Brain	Lungs	Heart	Spleen
Control	2.8±0.1	0.66±0.02	0.38±0.22	0.29±0.01	0.29±0.01	0.15±0.01
100mg/kg	2.9±0.1	0.66±0.02	0.40±0.01	0.31±0.02	0.30±0.01	0.16±0.01
200mg/kg	3.0±0.1	0.67±0.03	0.43±0.01	0.32±0.01	0.31±0.01	0.17±0.01

Values are mean ±SEM from 10 animals in each group

The RBC and coagulation parameters did not show any biologically or statistically significant differences between the rats treated or controls. However, slight decrease (but have no dose dependent effect) in lymphocytes and neutrophils were noted in rats treated while these data also fell within the reference values (Table 6).

Table 6 - Haematological Parameter.

Haematological parameter	Control	*Trial drug*	
		100 mg	200mg
Total R.B.C. count (×10^6 mm3).	9.09±0.15	8.133±0.3879^{ns}	7.783±0.2587^{ns}
Total W.B.C. Count (×10^3 mm3).	12.67±0.22	8.183±0.3705^{ns}	8.340±0.4157^{ns}
Haemoglobin (Hb) (g/dl)	15.61±0.36	11.42±0.4167^{ns}	11.19±0.4144^{ns}
Hematocrit (%).	44.21±1.01	41.83±1.537^{ns}	38.83±1.851^{ns}
Platelets (×103 mm3).	834.91±24.01	759.0±13.56^{ns}	963.3±33.22^{ns}
Lymphocytes (%).	84.7±1.32	74.58±2.800^{ns}	68.17±1.973^{ns}
Neutrophils (%).	20.6±0.65	17.23±0.4773^{ns}	18.83±0.9458^{ns}

Values are mean ±SEM from 10 animals in each group. ns no significant change When compared with untreated control group.

In haematological and biochemical examination, no parameters differed in male and female groups but neither of these appeared to be of toxicological significance as they were slightly upper or lower than those of the control groups.

Furthermore no dose related histo-pathological changes were observed. Gross examination in necropsy and at microscopic examination revealed no changes that attribute to the administration of drug (Figure 1).

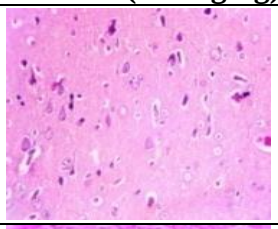
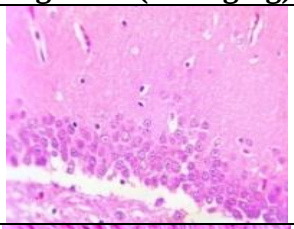
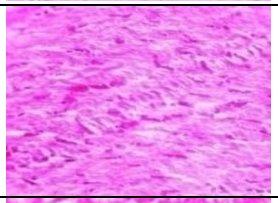
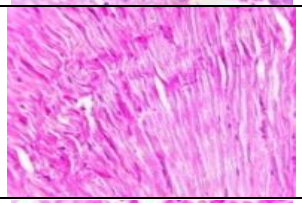
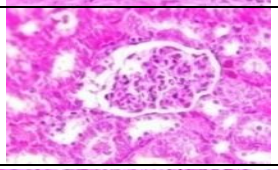
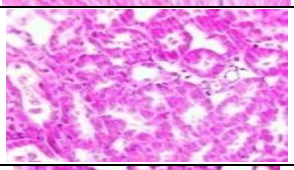
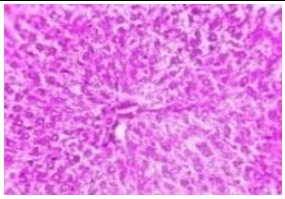
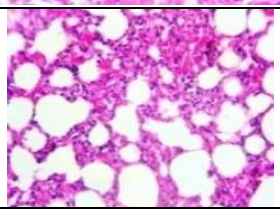
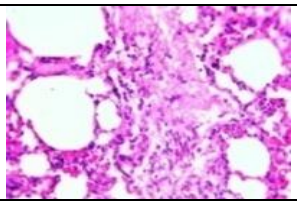
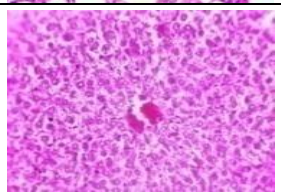
Organs	Low dose(100mg/kg)	High dose(200mg/kg)	Result
BRAIN			Cerebellum–Normal
HEART			Normal Cardiomyocytes
KIDNEY			Normocellular glomeruli
LIVER			Periportal zone - Normal
LUNG			Normal

Figure 1 - Histopathology of Vital Organs.

Compared with concurrent controls, rats fed with *Thalaga Kuligai* has not shown any changes in clinical chemistry and haematology values at 100 and 200 mg/kg and were regarded as non-toxic agents. ALT and AST are important serum enzymes in the human liver and usually helps detect chronic liver diseases by monitoring their concentrations.

In the present study, results showed that the concentration of ALT and AST were maintained regardless whether the rats were fed with *Thalaga Kuligai* or without in the treatment or

control groups. The organ-to-weight ratio is one of the fundamental judgments to diagnose whether the organ is exposed to the injury or not. Impaired organs often have abnormal tumidity or atrophy. There were no significant difference ($P > 0.05$) changes of organ-to-weight ratios in male and female rats or between treatment and control groups.

CONCLUSION

Thalaga kuligai was found to be safe in the acute toxicity study and with the results obtained the dose was fixed for the sub-acute toxicity study. Treating Wister albino rats with *Thalaga Kuligai* at levels of 100 and 200 mg/kg/day to male and female rats for 4 weeks has not caused any death. No toxicity induced abnormal values were observed in the general growth, body weight and organ weight, haematology and clinical chemistry values, nor did it cause any abnormalities in necropsy and histopathology findings.

Based on these results, *Thalaga Kuligai* could be categorized as no-observed-adverse-effect level (NOAEL) drug as it acts harmlessly under the current normal usage and this phenomenon is considered to be of no toxicological concern. The dosage 100 and 200 mg/kg/day to rats under the conditions of the sub-acute toxicity study have not revealed any signs or symptoms of toxicity. So *Thalaga kuligai* is considered non-toxic at these circumstances. Hence in this study to investigate the toxicity of *Thalaga kuligai* in rats in acute toxicity study model and in a 28-day oral toxicity trial, from the useful scientific knowledge obtained, we can conclude that this herbo-mineral drug is safe and can be efficiently implemented in the management of respiratory disorders.

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