



**EVALUATION OF SUB-ACUTE TOXICITY (ORAL) STUDY  
OF SIDDHA MEDICINE - SEENTHIL SARKKARAI IN WISTAR  
ALBINO RATS**

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**ABSTRACT**

Seenthil sarkarai has been used by the traditional medicine practitioners for the treatment of various diseases due to its rejuvenating and tridosha samani properties. The aim of the present study was to carry out sub acute oral toxicity studies of Seenthil

sarkarai in wistar albino rats. Seenthil sarkarai was prepared with the stem of Seenthil (*Tinospora cordifolia*) by traditional method. Twenty four wistar albino rats of either sex (3 male and 3 female) aging 6 to 7 weeks were selected based on the body weight and randomly distributed to 4 groups (Group I, II, III and IV). Distilled water was used as the vehicle and sub acute toxicity studies of Seenthil sarkarai were carried out by oral administration daily at doses of 360 mg/kg body weight, 1800 mg/kg body weight and 3600 mg / kg body weight in respective groups II- IV for 28 days. Vehicle Control group (Group-I) received distilled water only. During the study period, rats were observed weekly for toxicity symptoms, body weight gain and feed consumption. All surviving animals were euthanized on day 30th for hematological and biochemical analyses. No significant difference was observed with respect to body weight gain and feed consumption between the test groups and control group. No abnormal behavioral activity and preterminal deaths were recorded in the rats exposed to the test compound up to 10 times of the intended therapeutic dose. Thus the study demonstrates

that, short term (28 days) oral intake of Seenthil sarkarai causes no toxicity upto the dose of 3600mg / kg b.wt in wistar albino rats.

**KEYWORDS:** Siddha medicine, *Tinospora cordifolia*, Sub acute toxicity, Herb, 28 days toxicity.

## INTRODUCTION



**Figure 1:** *Tinospora cordifolia*.

There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics, etc because of the fact that the allopathic drugs have more side effects. Thus natural herbs, which are used traditionally in Indian system of medicine have become a safer alternatives to allopathic drugs.<sup>[1]</sup> *Tinospora cordifolia* commonly known as Seenthil is such a widely used herb in Siddha system of Indian medicine known for its analgesic<sup>[2]</sup>, anti-microbial<sup>[3]</sup>, anti-diabetic, hypolipidemic<sup>[4]</sup>, immunomodulatory<sup>[5]</sup>, anti-cancer<sup>[6]</sup>, anti oxidant<sup>[7]</sup>, anti-toxic<sup>[8]</sup>, anti-HIV<sup>[9]</sup>, anti-arthritis<sup>[10]</sup> and anti-osteoporotic<sup>[11]</sup> properties.

Seenthil sarkarai belonging to the family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude. There are three types of Seenthil namely common Seenthil, por Seenthil and paey Seenthil. Phytochemical investigation of extract of seenthil has revealed the presence of active compounds such as alkaloids, glycosides, lactones and steroids. All these active compounds have immunomodulatory and physiological roles of different types, thereby demonstrating the diverse versatility of the plant.<sup>[12]</sup>

Thus the aim of the present study was to carry out sub acute oral toxicity studies of Seenthil sarkarai in wistar albino rats.

## MATERIALS AND METHODS

### Plant Collection

Fresh samples of stem of Seenthil were collected from chengulput area in January 2011 and were identified and authenticated by the botanist Prof Jayaraman and voucher specimen was submitted.

### Preparation of Seenthil sarkarai

Seenthil sarkarai was prepared as per the text, Gunapadam porutpanbu -Mooligai vaguppu.<sup>[13]</sup> Seenthil stem were washed, outer covering of stem were removed, pounded and soaked in water and the soaked stem were squeezed and the juice was filtered. The juice was kept under the sun light for 3 hours and then the superannuated fluids were filtered; sediment deposited in the bottom was collected. The sediment was dried under sun light, and grounded in kalvam, then stored in air tight container.

### IAEC approval

The study protocol was approved during the Institutional Animal Ethics Committee meeting held on 08- 07-2011 at Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu. Proposal No. 113/PHARMA/SCRI, 2011 dated 08.07.2011.

### Experimental animals

A total of 24 Wistar albino rats of either sex aging 6 to 7 weeks, were received from Animal Breeding station, TANUWAS, Madhavaram, Chennai, Tamil Nadu. Only nulliparous and non-pregnant females were used in the experiment. Animals were housed 1 animal /cage in each polycarbonate cage with rice husk bedding and metal tops. Each cage was identified with cage card, which displayed study number, cage number, sex and animal identification numbers. Temperature and relative humidity were maintained at 18 to 25 °C and 30 to 65 % respectively and illumination was controlled to give approximately a sequence of 12 hours light and 12 hours dark. The animals were provided free access to autoclaved water purified with reverse osmosis and autoclaved standard pelleted laboratory animal diet *ad libitum* during the study period. Animals were acclimatized for 7 days before initiation of the study.

### Study protocol

Sub-chronic toxicity test was performed as per the guidelines of OECD guideline - 407 for the evaluation of safety of herbal medicines.<sup>[14]</sup> A total 24 animals (12 Males + 12 Females) based on the body weight were randomly distributed to 4 groups (Group I, II, III and IV).

Each group consisted of 6 animals (3 males and 3 females). Animals were distributed such that mean body weight variation will not be  $\pm 20\%$ . Group I received distilled water daily and Groups II, III, IV were orally administered with 360 mg/kg b.wt, 1800 mg/kg b. wt and 3600 mg /kg b.wt respectively of Seenthil sarkarai everyday for a period of 28 days. Distilled water was used as the vehicle in the current study as it is the most commonly used vehicle for oral toxicity studies. On 30<sup>th</sup> day, animals were over night fasted and all the biochemical estimations were performed. Blood was collected from retro orbital plexus under ether anesthesia. After biochemical evaluations, animals were euthanized and weights of major organs were measured followed by histopathological studies.

### Justification for Dose Selection

Doses selected for the present study were based on available Therapeutic human dose (TH) of Seenthil sarkarai i.e. 4000 mg per day.<sup>[13]</sup> The therapeutic dose was calculated by using dose conversion table.<sup>[15]</sup> The dose levels and groups were as follows.

**Table 1: Study protocol.**

Sr. no.	Group	Dose
1	I	Vehicle Control (VC) – Distilled water
2	II	Therapeutic Dose (TD) – 360 mg/kg
3	III	5 times Therapeutic Dose (5 TD) – 1800 mg/kg
4	IV	10 times Therapeutic Dose (10 TD) – 3600 mg/kg

### PARAMETERS MONITORED

Each animal in the four groups was observed for the physical appearances, behavioral abnormalities, mortality and morbidity twice daily and were recorded.

**Body Weight:** Body weight of each animal in the four groups was measured using an electronic weighing balance at the time of acclimatization, once before starting the experiment, weekly once during the experimental period and on the day of sacrifice after fasting the animals.

**Feed Consumption:** Feed consumption of animals was recorded weekly throughout the study. The food intake was quantified once daily by weighing the left over feed on standard electronic balance.

**Hematology:** The hematology parameters viz., total white blood cell count (WBC), total red blood cell count (RBC), hemoglobin concentration (HGB), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Platelet count (PLT) and differential leucocytes count (DLC) were analyzed.

**Biochemical parameters:** Creatinine (CREA), Glucose (GLU) and Total Protein (TP) were estimated using RA-50 auto analyzer (Bayer).

**Organ Weights:** Heart, liver, lungs, brain, kidney, eyes, stomach, intestine, pancreas, spleen, ovary and testis were taken from all surviving animals at the scheduled necropsies, weighed and recorded.

### STATISTICAL ANALYSIS

Statistical evaluation was performed using Graph Pad Prism Ver.5.0, for Windows XP. All results are presented as Mean±S.D. Data were analyzed using Student's 't'- test. Data were analyzed using one-way analysis of variance (ANOVA) and, when appropriate, by a Dunnett's pair wise comparison. Results were considered significant at  $p < 0.05$ .

### RESULTS

#### General signs

No deaths or significant changes in general behavior or other physiological activities were observed at any point in the present study.

#### Body weight and food intake

No significant changes were observed in body weight gain and food consumption in Seenthil sarkarai administered groups compared to vehicle treated group (Table 2 & 3).

#### Hematological and blood biochemical data

The hematological analysis (Table 4), showed no significant changes of RBC, WBC, Lymphocytes, Neutrophils, Hemoglobin (Hb), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Procalcitonin (PCT) in Groups II, III, IV when compared to Group I but levels of Monocytes and Platelets were slightly higher compared to control group but found to be within the normal range. The biochemical analysis (Table 5) showed no significant differences in Blood glucose, Serum creatinine, Albumin, Globulin, Serum glutamate pyruvate transaminase (SGPT), Sodium, Potassium and Chloride parameters examined in either the control or treated groups. A significant increase in Serum glutamate pyruvate transaminase (SGOT), Alkaline phosphatase (ALP) and cholesterol were seen in treated groups compared to vehicle control group.

**Organ weights**

There were no significant differences between the control and treated groups in the organ weights of rats (Table 6).

**Table 2: Body weight (weekly) of rats in sub acute toxicity of Seenthil sarkarai.**

S. No	Dose mg/kg	Initial.wt	I <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
1.	Control	102.5 ± 10.83	113.33 ± 10.32	126.66 ± 7.52	140.83 ± 5.84	150.83 ± 5.84
2.	360mg/kg	108.33 ± 14.71	115.83 ± 9.70	123.33 ± 7.52	133.33 ± 6.05	146.66 ± 4.08
3.	1800mg/kg	98.33 ± 14.71	105.83 ± 10.68	115.83 ± 8.01	129.16 ± 8.61	143.33 ± 6.83
4.	3600mg/kg	99.16 ± 14.28	110.83 ± 11.143	126.66 ± 7.52	135.83 ± 4.91	150 ± 4.47

n=6, Values are expressed as mean ± SD

**Table 3: Food Consumption (Weekly) of rats in sub acute toxicity of Seenthil sarkarai.**

S. No	Dose mg/kg	I <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
1.	Control	11.41 ± 1.50	12.11 ± 2.49	13.83 ± 2.55	14.33 ± 4.27
2.	360mg/kg	11.02 ± 1.09	14.33 ± 2.60	14.66 ± 1.63	13.95 ± 2.20
3.	1800mg/kg	10.66 ± 1.03	14.5 ± 1.51	15.16 ± 1.83	13.33 ± 1.63
4.	3600mg/kg	12.16 ± 2.40	11.33 ± 2.06	14.83 ± 2.51	15.5 ± 3.08

n=6, Values are expressed as mean ± SD

**Table 4. Hematological values of rats in sub acute toxicity studies of Seenthil sarkarai.**

S.No	Group	WBC	L	N	M	RBC	HGB	MCV	MCH	MCHC	PLT	PCT
1	Control	7.91 ± 1.14	64.2 ± 6.25	3.01 ± 0.56	32.78 ± 6.01	6.38 ± 0.82	10.63 ± 1.51	49.43 ± 1.57	16.58 ± 0.57	33.63 ± 0.70	219 ± 21.09	0.12 ± 0.01
2	360mg/kg	6.3 ± 0.91	53.58 ± 20.66	3.51 ± 0.37	42.46 ± 19.71*	6.79 ± 0.68	11.93 ± 1.15	53.2 ± 1.33	17.5 ± 0.57	33.65 ± 1.57	280 ± 41.46*	0.16 ± 0.02
3	1800mg/kg	8.06 ± 1.53	52.66 ± 13.64	3.78 ± 0.27	43.55 ± 13.57*	7.25 ± 0.48	12.65 ± 0.88	52.93 ± 1.40	17.38 ± 0.71	32.96 ± 0.66	300 ± 49.43*	0.17 ± 0.03
4	3600mg/kg	7.91 ± 3.18	69.98 ± 5.59	3.31 ± 0.41	26.7 ± 5.28	6.83 ± 0.76	11.86 ± 1.32	53.18 ± 1.55	17.31 ± 0.56	32.7 ± 1.02	255.5 ± 39.31*	0.14 ± 0.02

L= Lymphocytes, N = Neutrophils, M= Monocytes, RBC= Red blood cells, HGB= Hemoglobin, MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, PLT= Platelets, PCT= Procalcitonin  
n=6, Values are expressed as mean ± SD; \*P<0.05, compared to control.

**Table 5: Blood chemistry values of rats in sub acute toxicity of Seenthil sarkarai.**

S.No	Group	Blood glucose	Cholesterol	SCR	TPR	Alb	Glb	SGOT	SGPT	ALP	Na	K	Cl
1	Control	99.16 ± 11.30	44.33 ± 7.71	0.66 ± 0.13	6.65 ± 0.94	3.46 ± 0.23	3.81 ± 0.45	120 ± 16.03	80 ± 15.58	171.66 ± 80.42	128.66 ± 7.00	7.11 ± 0.66	105.16 ± 4.53
2	360mg/kg	104.33 ± 12.09	54.5 ± 9.26*	0.6 ± 0.08	8.01 ± 0.24	3.56 ± 0.23	4.45 ± 0.37	159.16 ± 9.30*	60.33 ± 19.29	263.5 ± 54.71*	127.73 ± 3.65	21.83 ± 3.66*	109.76 ± 2.50
3	1800mg/kg	122.83 ± 38.84*	65.33 ± 5.12*	0.5 ± 0.06	8.06 ± 0.29	3.53 ± 0.28	4.25 ± 0.24	147.5 ± 7.39*	55.83 ± 15.62	333 ± 47.92*	129.78 ± 8.93	16.58 ± 2.24*	107.48 ± 2.42
4	3600mg/kg	96.33 ± 6.47	94.16 ± 11.17*	0.51 ± 0.07	7.41 ± 0.55	3.46 ± 0.10	3.8 ± 0.45	139 ± 17.51*	54.16 ± 11.26	185.83 ± 51.66	134.2 ± 2.93	7.15 ± 0.28	107.76 ± 1.83

n=6, Values are expressed as mean ± SD ; \*P<0.05, compared to control

**Table 6. Organ Weights of rats in sub acute toxicity of Seenthil sarkarai**

S. No	Group	Heart	Liver	Lungs	Pancreas	Spleen	Kidney	Stomach	Intestine	Eyes	Brain	Testes	Ovary
1	Control	0.66 ± 0.06	5.3 ± 0.41	0.94 ± 0.09	0.12 ± 0.02	0.54 ± 0.05	1.44 ± 0.40	1.03 ± 0.15	5.50 ± 0.18	0.20 ± 0.02	1.10 ± 0.18	1.92 ± 0.19	0.2 ± 0.02
2	360mg/kg	0.70 ± 0.05	4.70 ± 0.44	1.03 ± 0.08	0.36 ± 0.41*	0.62 ± 0.05	1.5 ± 0.08	1.14 ± 0.11	6.37 ± 0.46	0.19 ± 0.01	1 ± 0.08	1.96 ± 0.15	0.19 ± 0.01
3	1800mg/kg	0.66 ± 0.06	4.80 ± 0.38	0.96 ± 0.12	0.61 ± 0.40*	0.58 ± 0.07	1.31 ± 0.23	1.05 ± 0.2	5.50 ± 0.42	0.19 ± 0.01	0.96 ± 0.08	1.78 ± 0.20	0.2 ± 0.01
4	3600mg/kg	0.65 ± 0.08	5.04 ± 0.40	0.95 ± 0.1	0.36 ± 0.41*	0.60 ± 0.05	1.15 ± 0.23	1.07 ± 0.07	5.31 ± 0.38	0.19 ± 0.01	1.03 ± 0.06	1.95 ± 0.05	0.19 ± 0.01

n=6, Values are expressed as mean ± SD; \*P<0.05, compared to control

## DISCUSSION AND CONCLUSION

The objective of the study was to determine toxicity profile, arising from repeated oral administration of Seenthil sarkarai to rats for a period of 28 days. The high dose of Seenthil sarkarai (3600 mg/kg day) in the sub acute study was applied because human exposure indicates the use of a high dose level. A lower dose of 360 mg/kg day was used to determine dose related toxic effects. In the study, it appeared that the Seenthil sarkarai at the doses used did not produce any marked changes in rats, as evidenced by the absence of toxic symptoms, no changes in water/food ingestion, or weight gain and survivability of animals until the scheduled euthanasia. On contrary, there were elevations in the levels of SGOT, ALP, total cholesterol and total proteins suggesting anabolic effect of the plant. The effect may be due to enhanced synthesis of certain modulator proteins in liver. Organ weight revealed that Seenthil sarkarai at the doses used, did not produce organ swelling, atrophy or hypertrophy apart from slight increase in weights of the pancreas. The comparable biochemical results in the control group and Seenthil sarkarai treated groups were consistent with the morphological analysis. In summary the Seenthil sarkarai was found to be nontoxic when rats were exposed to the test compound up to 10 times of the intended therapeutic dose in oral subacute toxicity studies.

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**Conflict of interest statement:** The authors report no conflict of interest.

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