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ACUTE AND CHRONIC TOXICITY STUDY ON SIDDHA SINGLE HERBAL MEDICINE "SEENTHIL SARKARAI"

Dr. Juliet Ruby*[1], T. Thiyagasundaram[2] and R. Manickavasagam[3]

[1]Lecturer, RVS Siddha Medical College, Sulur, Coimbatore, Tamilnadu, India.

[2]Lecturer, Sri Sairam Siddha Medical College, Tambaram, Chennai, Tamilnadu, India.

[3]Research Officer, Siddha Clinical Research Unit (Under Central Council for Research in Siddha), A and U Tibbia College and Hospital Campus, Karol Bagh, New Delhi, India.

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***Corresponding Author**
Dr. Juliet Ruby
Lecturer, RVS Siddha Medical College, Sulur, Coimbatore, Tamilnadu, India.

ABSTRACT

Awareness towards the traditional medical systems like Siddha, Ayurveda and Unani (ASU) is increasing day by day throughout the world. This awareness is increasing not only among educated population, also among the common public. permenant relief, no side effects and low cost are the reasons bring the towards the traditional system of medicines compare than allopathic system. Herbal based raw drugs are most commonly used in preparation of medicines in traditional medical system. In general belief, herbal based medicines are safe to consume and they can not produce any side effects. This theory of belief cannot accept by scientific community. Eventhough Herbal based Siddha medicines are safe and having the history of prolonged usages, It is our responsibility to clarifiy the safety measures of these medicines by using modern scientific parameters. Even in Siddha system, Siddhar mentioned suddhi process of herbals, before make them into medicines. Dose,Anupanam and duration of the Siddha herbal medicines are also mentioned by Siddhars. The aim of this study is to prove that "Seenthil Sarkarai" Siddha Single herbal preparation is safe, with proper dose and timeline as per mentioned in literature. Acute and Chronic toxicity studies are found to be safety, conducted in Lab animals.

KEYWORD: Siddha, Seenthil Sarkarai, Safety, Acute Toxicity, Sub-Acute Toxicity, Chronic Toxicity.

INTRODUCTION

Awareness towards the traditional medical systems like Siddha,Ayurveda and Unani (ASU)is increasing day by day throughout the world. This awareness is increasing not only among educated population, also among the common public. permenant relief,no side effects and low cost are the reasons bring the towards the traditional system of medicines compare than allopathic system. Herbal based raw drugs are most commonly used in preparation of medicines in traditional medical system.In general belief,herbal based medicines are safe to consume and they can not produce any side effects.This theory of belief cannot accept by scientific community. Eventhough Herbal based Siddha medicines are safe and having the history of prolonged usages, It is our responsibility to clarifiy the safety measures of these medicines by using modern scientific parameters.

Even in Siddha system, Siddhar mentioned suddhi process of herbals,before make them into medicines. Dose, Anupanam and duration of the Siddha herbal medicines are also mentioned by Siddhars.

Seenthil (Tinospora Cordifolia) is not classified under schedule E drugs- Poisionous herbal drugs category in Drugs and Cosmetics Act. A medicine prepared from Seenthil (Tinospora Cordifolia)-Seenthil Sarkarai is a Siddha classical Single herbal preparation which is an Extract taken by processing stem of Tinospora Cardifolia.It is indicated for the treatments of Polydypsia due to diabetes, JaundiceSkin diseases, Splenomegaly in Siddha Classical Text “Siddha Materia Medica, Gunapadam – Mooligai Vaghuppu”.

AIM OF THE STUDY

The aim of this study is to evaluate the safety profile of Siddha Single herbal Preparation “Seenthil Sarkarai”.

MATERIALS AND METHODS

Sources of Drug

The raw drugs were purchased from Raw drug shop–Somasundari Country drug Shop, Murukankurichi, Palayomkottai and auhtourized by pharmacognosist at Govt. Siddha Medical College Gunapadam Department.

Seenthil Sarkarai – Preparation

Remove the thin papery bark, cut the stem into pieces and crush in a stone mortar. Put the crushed drug in a large vessel, pour water and stir well. Next day remove from the water the coarse and fibrous part of the drug. Wash it in the water in the vessel. Repeat collecting, crushing and washing the fibrous material. Keep the water in the vessel for two to three hours until sedimentation occurs. Decant the clear supernatant water and collect the sedimented starch. Add some more water to this and remove if any more sediment of fibrous material is mixed with starch. Allow the starch to settle and carefully decant the water and collect the starch. Expose to sun and dry.

Dose: 4 gm. **Anupanam:** Milk. **Indication:** Fatigue due to fever, splenomegaly, jaundice, cough, vomiting, urinary tract infection.

Acute Toxicity Study

- **Animal Species:** Winstar albino rats bred in the animal house attached to the postgraduate, Department of pharmacology, Govt. Siddha Medical College of Palayamkottai were used.
- **Sex:** Animals of both sex were used.
- **Body weight:** Animals weighing between 80-120 gms.
- **Food and water:** The animals were maintained with standard animal feed and water adlibitum. The experimental animals were isolated and made accustomed to the lab conditions prior to the study.
- **Number of animals:** 30 rats were divided into 6 groups, each group consisting of 5 rats.
- **Dose levels:** The following dose levels were arbitrarily fixed by presuming a range of least toxic to highly toxic dose.

I Group	-	Control
II Group	-	200 mg / 100 g body weight of the animal.
III Group	-	400 mg / 100 g body weight of the animal.
IV Group	-	800 mg / 100 g body weight of the animal.
V Group	-	1600 mg / 100 g body weight of the animal.
VI Group	-	3200 mg / 100 g body weight of the animal.

- **Route of administration:** The drug was administered orally.

- **Preparation of the test drug for administration:** The drug was weighed and suspended with milk.(Suspending agent). It was ground well before administration. The preparation was done in such a way such that 1ml of suspension containing doses ranging from 200-3200mg of *Seenthil Sarkarai* which are given to the respective groups, as classified above in the doses level.

The drug was administered once, on the day of administration.

RESULT OF ACUTE TOXICITY STUDY

The said parameters in acute toxicity study were observed on various six groups (Group -I, Group - II, Group - III, Group-IV, Group-V, Group VI of which group-I is the control and the other groups were administered with the drug as follows 200mg /100 g bodyweight of the animal, 400 mg /100 g body weight of the animal, 800 mg /100 g bodyweight of the animal, 1600 mg /100 g bodyweight of the animal, 3200 mg /100 g bodyweight of the animal respectively. It is being found that the drug *"Seenthil Sarkarai"* didn't produce any mortality even upto 3200 mgm / 100 gm body weight of the animal.Very mild sign like sleep, sedation were observed only in animals treated with 1600 mg /animal and 3200 mg /animal (Group V & VI level) and that was even seen only after 24hrs.So it is inferred that the drug is safe upto 3200 mg / 100 gm body weight of the animal.There is no mortality rate at high dose level (3200 mg) and the histo pathology study of the animal is also carried out.

Chronic Toxicity

- **Introduction:** The *Seenthil Sarkarai* is used for treating the Polydypsia due to diabetes, Jaundice

Skin diseases, Splenomegaly, in Siddha system of Medicine. The drug is usually given upto 90 days. Since this drug is usually given for a long term in chronic ailments, it was decided to find the chronic toxicity of the drug in experimental animals.

- **Animals Species:** Winstar albino rats bred in the animal house, attached to the Postgraduate Department of Pharmacology, Govt. Siddha medical College, Palayamkottai were used.
- **Sex:** Animals of both sexes were used.
- **Body weight:** Animals weighing between 80 - 120 gms were selected.

- **Food and water:** The animals were maintained with standard animal feed and water adlibitum.
- **Number of animals:** 15 rats were divided into 3 groups, each consisting of 5 animals.

I. Selection of the dose levels: 2 S. doses were selected from the acute toxicity study. These doses did not have any acute toxicity effect and presumed to be safe for long term administration in animals.

Group - Control

Group - 200 mg / 100 gm body weight of the animal

Group - 400 mg /100 g bodyweight of the animal.

II. Preparation of the drug for administration: The drug was weighed and suspended with milk. It was ground well before administration. The preparation was done in such a way so as 1 ml of suspension contained 200mg and 400mg of *Seenthil Sarkarai* for the groups taken. The administration was done daily once in the morning.

Route of Administration: The drug was administered orally.

OBSERVATION AND RESULTS

Observation

The following details were recorded before the beginning of the drug administration.Weight of the animal,Haematological indices-a. WBC Total count b.WBC differential count c.Haemoglobin %. Every month the above parameters were recorded and they were also repeated at the end of the experiment. The results are tabulated. One Animal from each group were sacrificed at the end of the experiment and the visceras - Liver, Kidney, heart were removed from the animals and preserved in 40% formalin for histopathological studies.

Histopathological process

The sections were stained with haematoxylin and eosin and the histopathological report was given by Prof. Dr. V. Paramasivan H.O.D., Department of Pathology, Tirunelveli Medical college, Tirunelveli.

Table 1: Changes in the parameters of weight and hematological indices in Group I animals (Control).

S.No	Blood	At O' day (Mean)	At 3Oth day (Mean)	At 60th day (Mean)	At 90th day (Mean)
1.	WBC Total count	6200/cumm	6200/cumm	6500/cumm	6600/cumm
2.	Differential Count				
	Neutrophil	65%	75%	70%	70%
	Eosinophil	02%	-	02%	-
	Basophil	-	-	-	-
	Lymphocyte	33%	25%	28%	30%
	Monocyte	-	-	-	-
3.	Haemoglobin %	11g	11.2g	11.4g	11.5g
4.	SGOT	53IU/L	55IU/L	57IU/L	58IU/L
5.	SGPT	24IU/L	25IU/L	24IU/L	27IU/L
6.	Body Weight	100 g	100g	105g	105g

Table 2: Changes in the parameters of weight and haematological indices in Group II animals – 200mg/body weight of the animal.

S.No.	Blood	At O' day (Mean)	At 30th day (Mean)	At 60th day (Mean)	At 90th day (Mean)
1.	WBC Total count	7200/cumm	7200/cumm	7600/cumm	7800/cumm
2.	Differential Count				
	Neutrophil	60%	64%	62%	61%
	Eosinophil	01%	-	01%	02%
	Basophil	-	-	-	-
	Lymphocyte	39%	36%	37%	37%
	Monocyte	-	-	-	-
3.	Haemoglobin %	10.8g	11.2g	11.2 g	11g
4.	SGOT	57IU/L	56IU/L	57IU/L	57IU/L
5.	SGPT	22IU/L	21IU/L	22IU/L	23IU/L
6.	Body Weight	100 g	110g	130g	130g

Table 3: Changes in the parameters of weight and haematological indices in Group III animals – 400mg/body weight of the animal.

S.No.	Blood	At O' day (Mean)	At 30th day (Mean)	At 60th day (Mean)	At 90th day (Mean)
1.	WBC Total count	7100/cumm	7200/cumm	7400/cumm	7600/cumm
2.	Differential Count				
	Neutrophil	65%	66%	65%	68%
	Eosinophil	02%	-	01%	02%
	Basophil	-	-	-	-
	Lymphocyte	33%	34%	34%	30%
	Monocyte	-	-	-	-
3.	Haemoglobin %	11g	11.2g	11.2g	11.2g
4.	SGOT	61IU/L	59IU/L	59IU/L	62IU/L
5.	SGPT	25IU/L	23IU/L	27IU/L	26IU/L
6.	Body Weight	100 g	110g	115g	115g

Result of Chronic Toxicity Studies

The mean value of body weight and haematological indices for the three groups of rats (each group containing 5 animals with two different dosage levels were observed and the results were tabulated in tables I, II, III for the control, 200 mg/100 g bodyweight of the animal, 400 mg/100 g bodyweight of the animal dose groups respectively.

There is increase in the W.B.C count and Haemoglobulin in the haematological studies and also increase in the body weight of the animal.

Histopathological studies reveal that the *Seenthil Sarkarai* on long term administration produces pathological changes in the liver, kidney and the heart. So, the drug produces toxic effects on long term use.

Bio-Statistical Analysis

Probit Analysis

LD50 measurement (Toxicity)

1) If the test compound shows any pharmacological activity then the LD50 of the drug is determined.
2) By determining the LD50, we can justify whether to proceed with the drug or not.

Acute toxicity study analysis

Group	Dose in mg/ body weight of the animal	No. of Rats	No. of Rats died
I	200	5	-
II	400	5	-
III	800	5	-
IV	1600	5	-
V	3200	5	-

Since, there is no mortality of the animals in Acute Toxicity Study lethal dose of the drug could not be calculated.

Chronic toxicity study

Groups	Dose	No. of Rats	Days	No. of rats died
Group I	200	5	0	-
			30	-
			60	-
			90	-
Group II	400	5	0	-
			30	-
			60	-
			90	-

In case of Chronic Toxicity Study, with the help of physiological parameters such as Hematological investigations and with the histopathological studies the drug reaction with-in the animal can be assessed and are being tabulated respectively.

Lethal dose of the drug *Seenthil Sarkarai* can be calculated with higher dose level of the drug which can be done in further studies

DISCUSSION

The author went through the toxicity studies on albino rats for *Seenthil Sarkarai*.

The present study with *Seenthil Sarkarai* was conducted with an objective of finding out, whether this drug has got any side effects in long term administration to patient. *Seenthil Sarkarai* is a drug used to treat various chronic diseases such as Polydypsia due to diabetes, Jaundice, Skin diseases, Splenomegaly etc., The drug will have to be administered for a long duration of time depending upon the severity of the disease condition. So it was decided to study briefly the acute and chronic toxicity of *Seenthil Sarkarai*. While studying this drug experimentally, every precaution was taken, as it is administered. With this view, the drug was administered with proper adjuvant in all experiments conducted.The details of experiment have been already given. A brief outline of the same is given below for discussion.

Acute toxicity study

As per the findings of the study it is found that the single oral doses upto 3200 mg/100 g bodyweight of the animal, *Seenthil Sarkarai* did not produce any mortality, even at the end of 24 hrs. The drug produces mild sleep and sedation at the end of 24 hrs of drug administration.

Chronic toxicity study

As per findings of long term administration of *Seenthil Sarkarai* in the dose at the level of 200 mg /100gm body weight of the animal and 400 mg /100gm body weight of the animal produce sinusoidal congestion, prominent central vein and fatty changes in the liver, focal congestion and crowded nucleus in the heart, distended tubules with hypercellular glomerulus and degenerative changes in the kidney. These changes denoting the potential adverse effect of *Seenthil Sarkarai* in long term use. So the dose has to be reduced to smaller doses for the safety of patients while long time use in future.

On applying Bio-Statistical measures to the Acute and Chronic toxicity studies, the drug *Seenthil Sarkarai* is found to be safe upto 3200mg/body weight of the animal and the lethal dose of the drug *Seenthil Sarkarai* could not be calculated as there is no mortality of the animals taken for the study.

SUMMARY

The drug *Seenthil Sarkarai* was used by siddhars for the treatment of chronic diseases like Jaundice, Polydypsia due to diabetes, Skin diseases, Splenomegaly etc. The aim of this dissertation is to find out the acute and chronic toxicity of the drug *Seenthil Sarkarai* administered at various presumed moderate dose levels in the experimental animals.

For Animal Toxicity Study, the wistar albino rats with both sex were selected from animals house attached to the Government Siddha Medical College, Palayamkottai. The animal weight is between 80 -120 gms, were selected with standard food and water. To evaluate the acute toxicity study, 30 rats were selected and divided into 6 groups, each group consisting of 5 rats, and they were administered with the drug in different graded dose levels upto 3200 mg/100 gm body weight of the animal orally. The animals were observed and the details were recorded. The drug did not produce mortality even upto 24 hrs, so the drug is safe upto 3200 mg/100 gm body weight of the animal except mild sedation and sleep. The chronic toxicity study was conducted for about 90 days duration. In this study 2 dose levels were selected from acute toxicity study for the drug administration. Fifteen rats were selected and divided into 3 groups consisting of five rats. First group kept as control administered only with water.Second group was administered with *Seenthil Sarkarai* at the dose 200 mg/100 gm body weight of the animal and third with 400 mg/ 100 gm body weight of the animal respectively. These animals were sacrificed at the end of the experiment. The visceras - Liver, Kidney. Heart were removed from the animal and sent to the pathologist for histopathology report. The result revealed pathological changes in liver, Kidney and heart. In haematological studies there is no significant changes. The results are presented in tables and also affixing the relevant photos. These studies were discussed and concluded. The dose may further be reduced to smaller doses for the safety of the patients in long term use in future. Using Biostatistical Analysis, the lethal dose could not be calculated since no mortality was produced in Acute Toxicity Study.

This is initial toxicity study of *Seenthil Sarkarai*. It will be very useful for further research in future.

CONCLUSION

From the studies conducted we come to know that, *Seenthil Sarkarai* did not produce death in rats with in 24 hours at the dose level of 3200 mg/100 g body weight of the animal.

The chronic toxicity studies also revealed that the drug has harmful effect on liver kidney and heart in long term administration. The dose administered for chronic toxicity studies in rats are relatively very high, compared to the dose administered to the patients.

The aim of giving such a high dose was to find out the type of toxicity produced by it. This toxicity could occur in patient if the prescribed dose is not advised by the physician or not followed by the patient.

Further studies with smaller doses may perhaps establish the safety of the drug. In clinical practice, the drug *Seenthil Sarkarai* should be used with caution. The patient must be advised by the physician to follow correct dose (marunthalavu), course of treatment (naal alavu), adjuvant (anupanam) and diet restrictions (pathiyam). The physician should regularly monitor the patient by doing haematological examination and also the liver and kidney and the cardiac function tests.

Fig 1 - Liver Normal (Control)

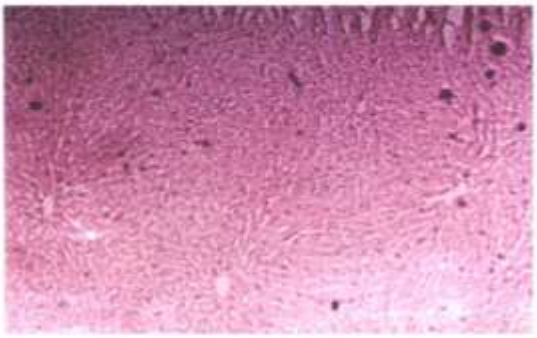


Fig 2 - Liver Normal (Control)

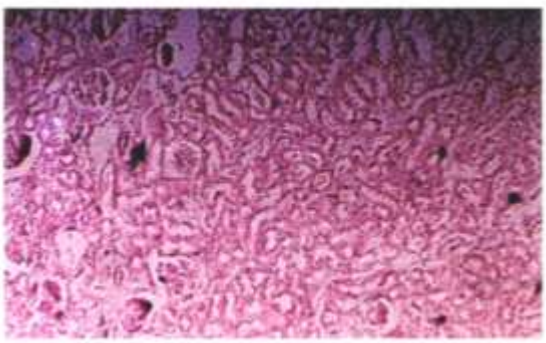


Fig 3 Heart - Normal (Control)

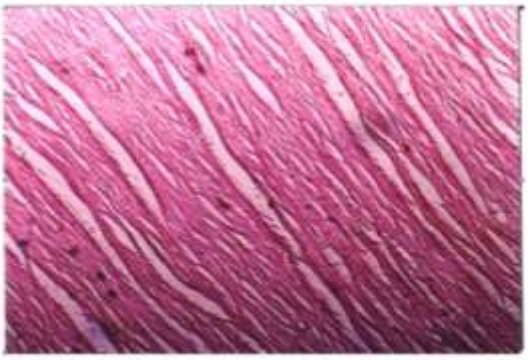


Fig 4 Liver - Shows Sinusoidal Congestion and Prominent Central Vein

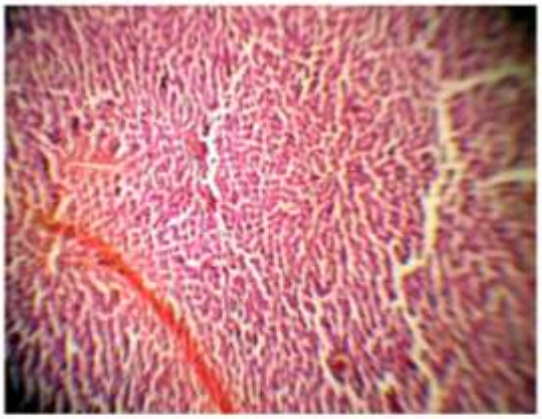


Fig 6 Liver - Shows Fatty changes

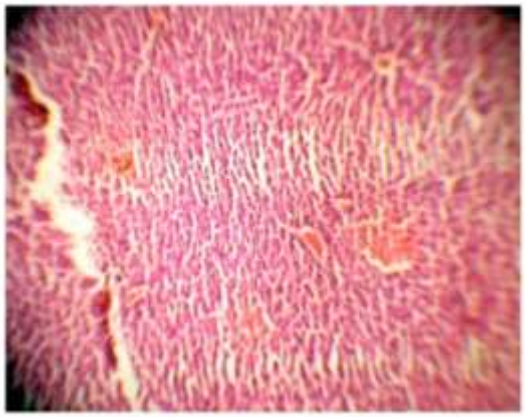


Fig 5 Kidney - Shows distented Tubules with Hyper cellular glomerulus

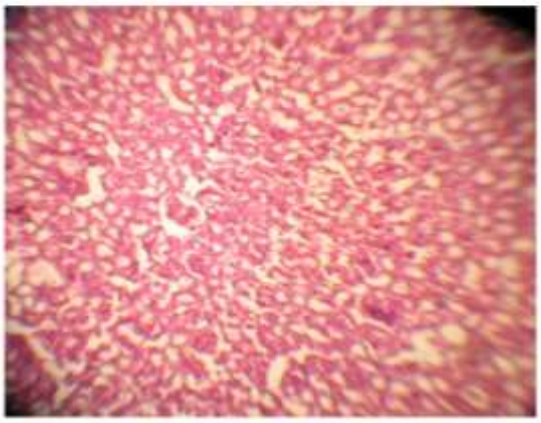


Fig 7 Kidney - Shows degenerative changes

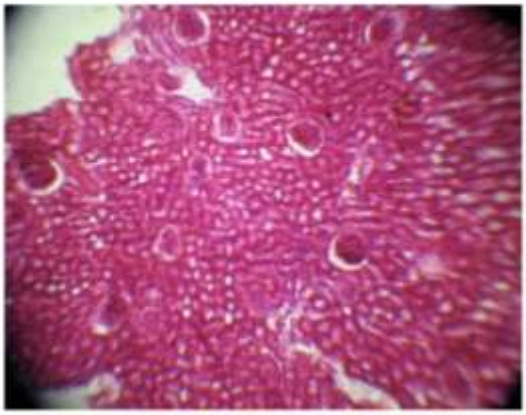
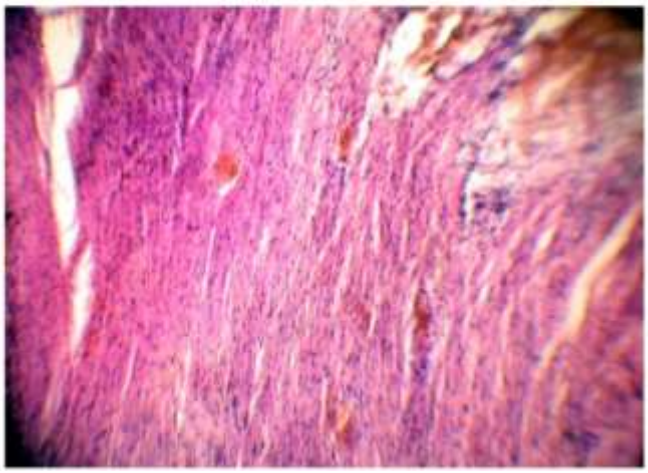


Fig 8 Heart - Shows Focal Congestion and Nucleus shows crowding



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