

TOXICOLOGICAL SCREENING OF A NOVEL SIDDHA POLYHERBAL FORMULATION "SIRINGIPAERATHI CHOORANAM"

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ABSTRACT

Objective: The aim of the present study is to validate the safety efficacy of "Siringipaerathi Chooranam" (SPC) in acute and sub-acute studies in the animal model.

Methods: Siddha system of medicine is the one of the earliest systems of medicine, which was practiced by our spiritual scientists. It constitutes plants, animals, metals and mineral formulations. Chooranam are fine to dry powders of drugs. Hence I have preferred to choose "Siringipaerathi Chooranam" (SPC) which is indicated for hepatoprotective activity and it was prepared as per the classical Siddha literature. The adult wistar albino rats were used for acute toxicity for 14 d and sub-acute studies for 28 d as per OECD guidelines 423 and 407. The test drug was made Suspension with 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. The drugs were orally administered to the dosage in the levels of 100, 200 and 2000 mg/dose in acute and subacute studies. The ingredients of SPC are *Inji* (*Zingiber officinalis*), *Milagu* (*Piper nigrum*), *Thippili* (*Piper longum*), *Thipili moolam* (Root of *Piper longum*), *Lavanga pathiri* (*Cinnamomum tamala*), *Elam* (*Elettaria cardamomum*), *Kodiveli ver* (*Plumbago zeylanica*), *Lavanga pattai* (*Cinnamomum zeylanicum*), *Moongil uppu* (*Bambusa arundinaceae*), *Sandhana thool* (*Santalum album*), *Vilamichu-ver* (*Plectranthus vettiveroides*), *Sathikkai* (*Myristica fragrans*), *Seeragam* (*Cuminum cyminum*), *Kirambu* (*Syzygium aromaticum*), *Sugar* (*Saccharum officinarum*), *Nei* (Ghee).

Results: The present investigation shows that there were no significant toxicity changes seen during the study. The body weight, food, water intake, behavioral, CNS, ANS, CVS, Vitals, Hematology, Biochemical and Histopathology of kidney, liver, spleen were observed both in control and test group animals were appears to be normal range.

Conclusion: Thus the authors conclude from the results that the safety efficacy of SPC through acute and sub-acute toxicity studies in rodents.

Keywords: Siddha, Siringipaerathi Chooranam, SPC, Toxicity, OECD, Poly Herbal Formulation

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INTRODUCTION

Siddhars are the spiritual scientists, who established the Siddha science. Siddhars have more knowledge about the universe and its contents. The main goal of Siddha system of medicine is to satisfy the people with a healthy and hygienic life. "Pancha bootham" (Five primordial elements) and "Tridhosam" (Three humors) forms the basis of Siddha. All the physiological function in the body is arbitrated by "Tridhosam" ('Vatham', 'Pitham' and 'Kabam') [1].

Liver is the largest organ in a human body, situated in the right side of the upper abdominal cavity. It plays an important role in Carbohydrate, Protein, Lipid, Bilirubin, Bile acid, Vitamin and Mineral, Hormone, Drug, Alcohol, and Cholesterol metabolism. The liver plays a vital functions in the maintenance, performance and regulating homeostasis of the body. The bile secreted by the liver plays an important role in digestion [2].

Environmental pollutions, fast foods, drugs, alcohol, and sedentary lifestyles are the causes of Liver diseases. The most common diseases of liver includes Hepatitis, Alcohol-related liver diseases, Liver tumor and Rey's syndrome [3].

Liver disease is the major cause for its morbidity and mortality in public affecting humans of all ages. This organ is called as metabolic hub because all the mechanisms take place in it. So, it's our duty to preserve this organ without any toxicity. Thus I prefer to screen toxicity studies before validating the pharmacological activity of polyherbal formulation "Siringipaerathi Chooranam".

So we have to make the most of the existing formulations or evaluating new treatments. Now-a-days there are so many medicines have been used in the treatment of liver diseases, but there is a need for the drug which could be less toxic, cost-effective and more potent. Among them,

one of the polyherbal formulation mentioned in Siddha literature for hepatoprotective activity is "Siringipaerathi Chooranam" consists of *Piper longum*, *Cuminum cyminum*, *Syzygium aromaticum*, *Bambusa arundinacea* and *Cinnamomum tamala* which acts as a Hepatoprotective and Anti-oxidant activity.

MATERIALS AND METHODS

Drug selection

In this research purified and prepared "Siringipaerathi Chooranam" was taken as a trial drug for Hepatoprotective activity from the Siddha literature "Sarabendra Vaidhiya Muraigal". Soolai, Moola, Kusta, Pitharoga Muraigal, page no: 194-195.

Collection of the plant materials

All the raw materials were bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug

All the plant materials were identified and authenticated by the Botanists and Gunapadam experts in Government Siddha Medical College, Arumbakkam, and Chennai-106. The specimen sample of all the herbs have been preserved in PG Gunapadam department individually for future reference.

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature [4].

Inji-Outer skin of ginger was peeled off.

Milagu-It was soaked in sour buttermilk for 3 h and allowed to dry

Thippili-Soaked in lemon juice and allowed to dry.

Thippilimoolam-Remove the nodules and dried.

Lavangapathiri-Dried in sunlight.

Elam-Roasted in the pan and outer skin was removed.

Kodiveli-ver-The root was cleaned with a white cloth.

Lavangapattai-Dried in sunlight.

Sandhana kattai-The skin was peeled off to get purified and powdered

Vilamichu-ver-The root was cleaned with a white cloth.

Sathikkai-Cleaned and cut into small pieces and dried.

Seeragam-Clean the dust particles and allowed it to dry.

Kirambu-Flower buds were removed.

Table 1: Ingredients

Name of drugs	Botanical name	Quantity
<i>Inji</i>	<i>Zingiber officinalis</i>	560 gm (16 palam)
<i>Milagu</i>	<i>Piper nigrum</i>	50.4 gm (12 varahan)
<i>Thippili</i>	<i>Piper longum</i>	33.6 gm(8 varahan)
<i>Thippilimoolam</i>	<i>Piper longum</i>	16.8 gm(4 varahan)
<i>Lavanga pathiri</i>	<i>Cinnamomum tamala</i>	35 gm(1 palam)
<i>Elam</i>	<i>Elettaria cardamomum</i>	42 gm (10 varahan)
<i>Kodiveli ver</i>	<i>Plumbago zeylanica</i>	42 gm (10 varahan)
<i>Lavanga pattai</i>	<i>Cinnamomum zeylanicum</i>	35 gm (1 palam)
<i>Moongil uppu</i>	<i>Bambusa arundinaceae</i>	35 gm (1 palam)
<i>Sandhana thool</i>	<i>Santalum album</i>	35 gm (1 palam)
<i>Vilamichu-ver</i>	<i>Plectranthus vettiveroides</i>	35 gm (1 palam)
<i>Sathikkai</i>	<i>Myristica fragrans</i>	35 gm (1 palam)
<i>Seeragam</i>	<i>Cuminum cyminum</i>	35 gm (1 palam)
<i>Kirambu</i>	<i>Syzygium aromaticum</i>	35 gm (1 palam)
<i>Sugar</i>	<i>Saccharum officinarum</i>	Equal quantity
<i>Nei</i>	English Name: Ghee	30 ml

Preparation of the drug

Procedure

In order to obtain the purified form of ginger, the upper skin of ginger was peeled off and then sliced into small pieces. The sliced pieces were dried in sunshade for two days. After complete drying 560 grams of dried ginger was taken and fried well in ghee and then powdered.

50.4 grams of Purified Pepper, 33.6 grams of *Thippili*, 16.8 grams of *Thippilimoolam*, 42 grams of *Kodiveli-ver*, 35 grams of *Moongil uppu*, *Lavangapathiri*, *Sandhana thool*, *Vilamichu-ver*, *Lavanga Pattai*, *Adhikari*, *Seeragam*, *Kirambu* were taken and powdered separately then mixed together with processed ginger powder.

Finally, the mixture was ground well which favors the homogenous preparation. Then the mixture powder was sieved through the thin clean white cloth. After that, twice the weight of sugar was added to the mixture and again it was ground well.

Finally, the end product was obtained, which was kept in an airtight container and labeled as "*Siringipaerathi Chooranam*" (SPC).

Purification of the chooranam: steaming process (*Pittaviyal murañ*)

The "*Siringipaerathi Chooranam*" was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. A mud pot was taken and it was half filled by milk and mixed with an equal quantity of pure water. The mouth of the pot was sealed by a cloth. This *chooranam* was placed over a clean dry cloth and tied firmly around the mouth of the mud pot. The gap between mud pots was tied with wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk reduced in the lower pot.

The same drug was later dried and powdered then sieved again. It was used for further study [5].

Storage of the drug

The prepared test drug was stored in a clean, airtight glass container.

Administration of the drug

Form of the medicine: *Chooranam*

Route of Administration: Enteral

Dose: 2 gm twice a day depending on the severity

Adjuvant: honey

Indication

Kamaalai, *Marbuvali*, *Kirani*, *Suram*, *Vaanthi*, *Peenisam*.

Toxicological studies

Acute oral toxicity–OECD guidelines-423

As per the OECD guideline, acute toxicity is done. (Organization for Economic Co-operation and Development, Guideline-423 [96].

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval no: IAEC/XLIV/ 31/CLBMCP/2014.

Animal: Healthy Wistar albino female rat weighing 200–220 gm

Studied carried out at three female rats under a fasting condition, signs of toxicity were observed for every one hour for the first 24 h and every day for about 14 d from the beginning of the study.

Principle

It is the principle of test which is based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained in acute toxicity for a test substance to enable its classification. The substance is given to a group of experimental animals in the oral route at one of the defined doses. A stepwise procedure was done in testing the substance, each step using three animals of a single sex.

Presence or absence of compound-related mortality of animals which is dosed at one step will determine the next step, i.e.; – no further testing is needed with the same dose three additional animals are taken– dosing of three additional animals at the next higher or the next lower dose level. The method will be a judgment with respect to classify the test substance to one of its series to the toxicity classes.

Methodology

Selection of animal species

The preferred rodent species was rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house

of king's institute, Guindy, Chennai. Female should be nulliparous and non-pregnant.

Each animal at the commencement of its dosing should be between 8 and 12 w old and its weight should fall in an interval within $\pm 20\%$ of the mean weight of the animals. The studies were conducted in the animal house of C. L. Baid Metha College of pharmacy, Duraipakkam, Chennai.

Housing and feeding conditions

Temperature in this experimental animal room should be at 22 °C (± 3 °C). Even though the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting must be artificial, the sequence being 12 h light, 12 h dark. For feeding, laboratory diets might be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 d prior to dosing to allow for acclimatization to the laboratory conditions.

Experiment procedure

Administration of doses

"Siringipaerathi Chooranam" prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavages using a feeding needle. Animals were fasted prior to dosing. During the period of fasting, the animals were weighed and then the test substance was administered. After the substance which was administered, food was given for a further 3-4 h.

The principle of laboratory animal care was followed. Observations were done with recorded systematically and continuously observed as per the guideline after substance administration. Visual observations Such as skin changes, mobility, and aggressiveness, sensitivity to sound and pain, as well as respiratory movements. Food was deprived to the animals, but not water 16-18 h prior to the administration of the test suspension.

At last, the number of survivors was noted in 24 h and these animals were then maintained for 14 d and observations made daily. The toxicological effect was screened on the basis of mortality.

Number of animals and dose levels

Since this test drug has been under practice for a long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study: 48 h

Evaluation: 14 D

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

1. The animals were observed individually after dosing at least once during the first 30 min and periodically during the first 24 h.
2. Special attention: First 1-4 h after administration of the drug, and
3. It was observed daily thereafter for a total of 14 d, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 d.

Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study.

Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were done.

Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes an examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes, and uterus of all animals

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, a number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing "*Siringipaerathi Chooranam*" with 2% CMC solution and it was found suitable for dose accuracy.

Justification for the choice of vehicle

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique [6].

Repeated dose 28 D oral toxicity study Of "*Siringipaerathi Chooranam*" On rats-(OECD-407 Guidelines) [7]

Justification for dose selection

The results of acute toxicity studies in Wistar albino rats indicated that "*Siringipaerathi Chooranam*" was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were 100 mg/kg and 200 mg/kg body weight. The oral route was selected for use because the oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose

"*Siringipaerathi Chooranam*" at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 mg/kg. The test substance suspensions were freshly prepared every day for 28 d. The control animals were administered vehicle only. Administration was by oral (gavages), once daily for 28 consecutive days.

Methodology

Randomization, numbering and grouping of animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 d. Animals were allowed acclimatization period of 7 d to laboratory conditions prior to the

initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

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, at weekly 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 the entire course of study.

Functional observations

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory investigations

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as an anticoagulant. Blood samples were centrifuged at 3000 r. p. m. for 10 min. On 28th day of the experiment, 24h urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations.

On 29th day, the animals were fasted for approximately 18 h, and 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 min to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

RESULTS AND DISCUSSIONS

Toxicity study results

Table 2: Dose-finding experiment and its behavioral signs of toxicity for *Siringipaerathi* Chooranam observation done

Group	Day
Body weight	Increased
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urine	Normal

Haematological investigations

Blood samples of control and experimental rats were analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, Creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/Alanine aminotransferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis

Urine samples were collected on the end of treatment for the estimation of normal parameters. The estimations were performed using appropriate methodology.

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, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

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

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (3-5 µm 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 technique 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 heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals, were preserved they were subjected to histopathological examination [8].

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's multicomparison test using a computer software programme GRAPH PAD INSTAT-3 version.

Table 3: Dose finding experiment and its behavioral signs of toxicity for *Siringipaerathi Chooranam*

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. TouchResponse 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. Diarrhea 18. Writhing 19 Respirations 20. Mortality

Interpretation

In the acute toxicity study, the rats were treated with different concentration of *SiringipaerathiChooranam* from the range of 5 mg/kg to 2000 mg/kg which did not produce signs of toxicity, behavioral changes, and mortality in its test groups are compared

with the controls when observed during 14 d of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In an acute toxicity test, the *SiringipaerathiChooranam* was found to be non-toxic at the dose level of 2000 mg/kg body weight.

Weight gain of rats

Table 4: Body weight (g) changes of rats when exposed to SPC

Dose (mg/kg/day)	Days				
	0	7	14	21	28
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
100	121.53±0.93	124.14±0.58	127.24±1.15	128.92±1.40	132.23±1.05
200	122.65±0.91	127.83±0.90	128.23±1.15	130.59±1.59	134.05±0.98

Values are mentioned in mean ± SEM; N=10; *P<0.05, **P<0.01, ***P<0.001 vs control.

Interpretation

The total body weight of the animals was weighed on 1st, 7th, 14th, 21st, 28th day and is shown in the table. It was found that the test drug produced significant weight gain than control, with the administration of the drug. Similarly, the test drug at all dose levels

induced weight gain and we could see longer the duration of administration of drug higher was the weight gain.

Interpretation

There is a slight significant change in the organ weight of the rats treated with different doses of test drug and the control.

Results of organ weight in rats

Table 5: Effect of *Siringipaerathi Chooranam* on organ weight in rats

Organ	Control	100 mg/kg	200 mg/kg
Liver (g)	3.07±0.20	4.41±0.32	4.72±0.32
Heart (g)	0.32±0.04	0.37±0.01	0.42±0.02
Lung (g)	0.28±0.05	0.32±0.01	0.38±0.01
Spleen (g)	0.74±0.07	0.68±0.17	0.78±0.08
Brain (g)	0.37±0.05	0.47±0.02	0.54±0.03
Kidney (g)	0.76±0.05	0.89±0.01	0.91±0.02

Values are mentioned in mean ± SEM; N=10; *P<0.05, **P<0.01, ***P<0.001 vs control.

Results of haematological parameters

Table 6: Effect of *Siringipaerathi Chooranam* on haematological parameters in rats

Parameter	Control	100 mg/kg	200 mg/kg
RBC(x 10 ⁶ /mm ³)	8.29±0.43	8.27±0.44	8.26±0.44
PCV (%)	49.66±0.77	48.98±1.04	48.20±0.91
Hb (%)	15.13±0.39	14.83±0.40	15.03±0.39
WBC(x 10 ³ /mm ³)	11.75±0.85	11.73±0.85	11.74±0.85
Neutrophils (%)	23.29±0.73	21.94±1.03	22.96±0.49
Eosinophils (%)	4.10±0.23	3.8±0.25	3.97±0.23
Lymphocyte (%)	85.5±0.46	83.7±0.72	84.87±0.32
Platelets(x 10 ³ /mm ³)	425.73±1.35	423.43±1.47	425.03±1.26

Values are mentioned in mean ± SEM; N=10; *P<0.05, **P<0.01, ***P<0.001 vs control.

Interpretation

The haematological investigation results of the rats conducted on 28th day after the repeated dose of the drug revealed the values of

different parameters. There is a slight variation in the values of RBC count values in the dose group of 100, 200 when compared with that of the control. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Results of biochemical parameters

Table 7: Effect of *Siringipaerathi Chooranam* on biochemical parameters in rats

Parameters	Control	100 mg/kg	200 mg/kg
Protein (g/dl)	8.58±0.68	7.56±0.61	6.76±0.44
Albumin (g/dl)	5.34±0.40	5.29±0.44	3.5±0.54
BUN (mg/dl)	22.06±1.55	22.72±1.9	25.53±1.8
Urea (mg/dl)	64.24±3.11	66.7±5.3	69.2±2.9
Creatinine (mg/dl)	0.85±0.07	0.6±0.24	0.71±0.25
Total Cholesterol (mg/dl)	93.21±1.16	92.17±1.13	91.53±1.35
Triglycerides (mg/dl)	52.58±1.56	52.16±1.3	52.93±1.7
Glucose (mg/dl)	108.63±0.81	107.97±1.12	109.4±0.51
Total Bilirubin (mg/dl)	0.205±0.04	0.16±0.08	0.10±0.03
SGOT (U/l)	73±2.4	72.67±1.64	65.52±2.4
SGPT(U/l)	28.4±1.2	25.77±0.64	23.99±0.70
Alkaline phosphatase(U/l)	102.4±3.6	101.3±1.5	91.33±4.26

Values are mentioned in mean ± SEM; N=10; *P<0.05, **P<0.01, ***P<0.001 vs control.

Interpretation

The biochemical investigations were conducted on 28th day and the results are produced. The results revealed that there is a slight

significant change in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits.

Sub-acute oral toxicity 28 d repeated dose study in rats

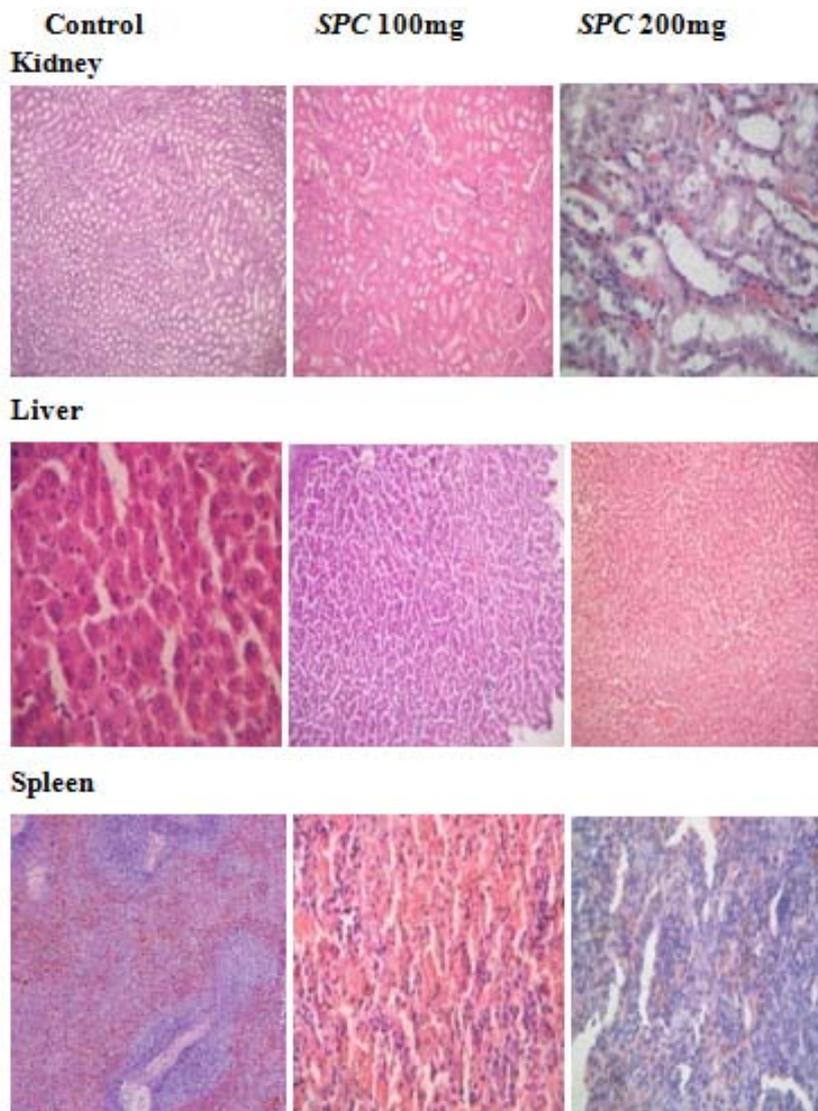


Fig. 1: Histopathology slides

Interpretation

The above slides show the histopathology studies of sub-acute toxicity. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Siringipaerathi Chooranam*. Thus the safety of the drug is revealed so that it can be administered for a long time without any side effects.

CONCLUSION

Liver diseases are the most common health problem in the world. The Liver is quantitatively the most important site of drug metabolism. However many drugs are known to cause hepatic injury. Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have a serious adverse effect. In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations from Siddha system of medicine recommended for the treatment of liver disorders. In order to overcome this difficulty in this paper, the authors facilitate a novel attempt to standardize the Siddha drug *Siringipaerathi Chooranam* for its hepatoprotective properties a toxicological screening was done as per the format of preclinical studies.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

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