INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE
ISSN: 2455-944X
www.darshanpublishers.com
DOI:10.22192/ijcrbm Volume 3, Issue 5 - 2018

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2018.03.05.002

Acute and chronic toxicity study of Silasathu parpam on Wister albino rats

Vikesh. B[1], Radha. S[2], Thanikaiselvi. S[3], Thiruthani. M[4]

[1]PG scholar, Department of Nanju Noolum Maruthuva Neethi Noolum, Govt Siddha Medical College, Palayamkottai.
[2]Reader, Department of Nanju Noolum Maruthuva Neethi Noolum, Santhigiri Siddha Medical College, Tiruvandrum.
[3]PG scholar, Department of Gunapadam, Govt Siddha Medical College, Palayamkottai.
[4] Head of the Department of Nanju Noolum Maruthuva Neethi Noolum, Govt Siddha Medical College, Palayamkottai

Abstract

Background: Silasathu Parpam (SP), a Siddha medicine used for the treatment of Urinary tract infections. Present study was undertaken to demonstrate the oral toxicity of SP in Wister albino rats. Methods: Acute and 90-days repeated oral toxicity studies were performed following OECD test guidelines 423 and407, respectively. In acute oral toxicity study, SP was administered at 200, 400, 800, 1600, 3200 mg/100gm and animals were observed for toxic signs at 1, 2, 4, 24 hours and for next 14 days. Gross pathology wasperformed at the end of the study. In repeated dose, the 90- days oral toxicity study, SP was administered at 200 and 400 mg/100gm. Three groups were also maintained to determine the delayed onset toxicity of SP. 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 and histopathology was done in organs Results: In acute toxicity study, no treatment related death or toxic signs were observed with SP administration. In the repeated dose study, no significant differences in body weight changes, haematology, were observed between control and SP groups. No gross pathological findings and difference in relative organ weights were observed between control and SP treated rats. Histopathological examination revealed no abnormalities with SP treatment. Conclusion: Silasathu Parpam had not produced any mortality or signs of toxicity even up to a dose level of 3200mg/100gm body weight in acute toxicity study. The drug possesses very low toxicity on long term administration due to high dosage. The doses selected for toxicity studies were relatively higher when compared to the clinical dose. Since this study reveals that the therapeutic dose for silasathu parpam mentioned in the text could be concluded safe for clinical practice.

Keywords: silasathu parpam, acute toxicity, chronic toxicity, siddha

Introduction

Siddha formulations were either purely herbal or mineral or metal or a combination of herbomineral or metals. Medicines prepared from minerals and salts have long shelf life than herbals and are often used for chronic diseases. The silasathu parpam is a mineral and salt combination used for treating Urinary Tract Infection. Dose and duration of administration of drug is much more important for clinical practice. It is the duty of a physician to ensure that the patient is receiving the correct dose and is harmless. Evaluation on safety aspects using scientific parameters is essential in growing scientific world. A scientific evaluation is the need of the hour for this common medicine which is used extensively. Present study was

undertaken to demonstrate the oral toxicity of Silasathu Parpam in Wister albino rats.This study will proved a scientific evidence for the safety of Silasathu Parpam to be used clinically.

Materials and Methods

The procedures of pre-drug preparation and drug preparation were strictly based on siddha literature. Raw drugs were purchased at Nagercoil, Tamil Nadu, India through proper identification.

Ingredients of silasathu parpam:

KarpooraSilasathu (Selenite)
Padikaram (Alum)
Vengaram (Borax)
Indhuppu (Rocksalt)
Vediuppu (Potassium nitrate)

Purification of Raw Drugs:

1. Karpoorasilasathu:

Karpoorasilasathu was boiled in milk and washed with water then dried

2. Padikaram:

Padikaram was made into fine powder and dissolved in water. The solution was filtered by using a clean cloth and the impurities were removed. The solution was then subjected to heat till it becomes viscous (kulambupatham) and dried.

3. Venkaram:

Venkaram was placed in a mud pan and roasted till the moisture is removed.

4. Indhuppu:

Indhuppu was soaked in vinegar for about three days and then dried in sunlight

5. Vediuppu:

Sea water were taken in the ratio 2:1 and mixed well. The solution was heated till it boils and dried in sunlight. The process was repeated for about seven times.

Method of preparation:

The purified drugs were taken in the following ratio

Karpoorasilasathu	: 4 varagan (16.4 gm)
Padikaram	: 1 varagan (4.1 gm)
Venkaram	: 1 varagan (4.1 gm)
Indhuppu	: 1 varagan (4.1 gm)
Vediuppu	: 1 varagan (4.1 gm)

The drug were powdered separately and mixed well. The mixture was kept in a mud pan, covered by using another mud pan and sealed with clay. It was dried in sunlight and subjected to pudam with fifty cow dung cakes (varaties). Then it was allowed to cool and the final product was collected and ground into fine powder. The powder was filtered through a piece of clean cotton cloth.

Adjuvant:

Honey

Indication:

Moothira Kiricharam (Urinary Tract Infection).

Animals

Wister Albino rats of either sex, weighing 80 gm to 120 gm were used from post graduate pharmacology department, Govt Siddha Medical College, palayamkottai. The animals were feed on standard rodent pellet and RO water was provided *ad libitum*. The animals were kept for overnight fasting before experimentation. All experimental procedures described were reviewed and approved by the Institutional Animal Ethical Committee of Govt Siddha Medical College, palayamkottai and the IAEC approval no. **040/IAEC/GSMC/2011-2012.**

Acute oral toxicity OECD 423 guidelines

Acute toxicity studies were carried out according to the OECD (Organization of Economic Cooperation and Development) guidelines 423. Healthy female rats, weighing 80–120gm, were selected and oral administration of the single doses of Silasathu Parpam were done.

Administration of doses:

Silasathu parpam was suspended in equal amount of honey and water administered as a single oral dose by using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance 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 guideline after substance administration. The control animals were administered vehicle only. The test substance was administered as oral dose of 200 mg, 400 mg, 800 mg, 1600 mg and 3200 mg per 100gm body weight step by step according to the guidelines. The general behaviours of rats were continuously monitored for 1 hours 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 signs of toxicity or mortality were recorded. The visual observations included Stimulation (Hyperactivity, Pyloerection, Twitching, Rigidity, Irritability, Jumping, Colonic convulsions, and Tonic convulsions) Depression (Ptosis, Sedation, Sleep, Loss of Traction, Loss of pinna reflex, Ataxia, Loss of muscle tone, Analgesia) Autonomic effect (Straub tail, Laboured respiration, Cyanosis, Reddening, Abnormal secretions) and Mortality. They were deprived of food, but not water 12 hours prior to the administration of the test substance. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Repeated dose 90-day chronic oral toxicity study (OECD – 407guidelines)

Chronic toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female).The control animals were administered vehicle only. Silasathu Parpam was administered to rats at the dose of 100 & 200 mg/100gm/day for 90 days. 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 and histopathology was done in organs like Liver, Heart, Kidney, and Brain.

Justification for Dose Selection:

The result of acute toxicity studies in Rats indicated that Silasathu Parpam was nontoxic and no behavioural changes were observed up to the dose level of 3200 mg. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose:

Silasathu Parpam three doses respectively were suspended with honey and water. It was administered to animals at the dose levels of 100 and 200 mg/100gm. The test substance suspensions were freshly prepared every day for 90 days. The control animals were administered vehicle only. Administration was done by oral once daily for 90 consecutive days.

Laboratory Investigations:

Following laboratory investigations were carried out on 91-th day in animal fasted over-night. On91th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with etherand 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 analysed for biochemical parameters.

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technician 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 histopathological examination.

Results

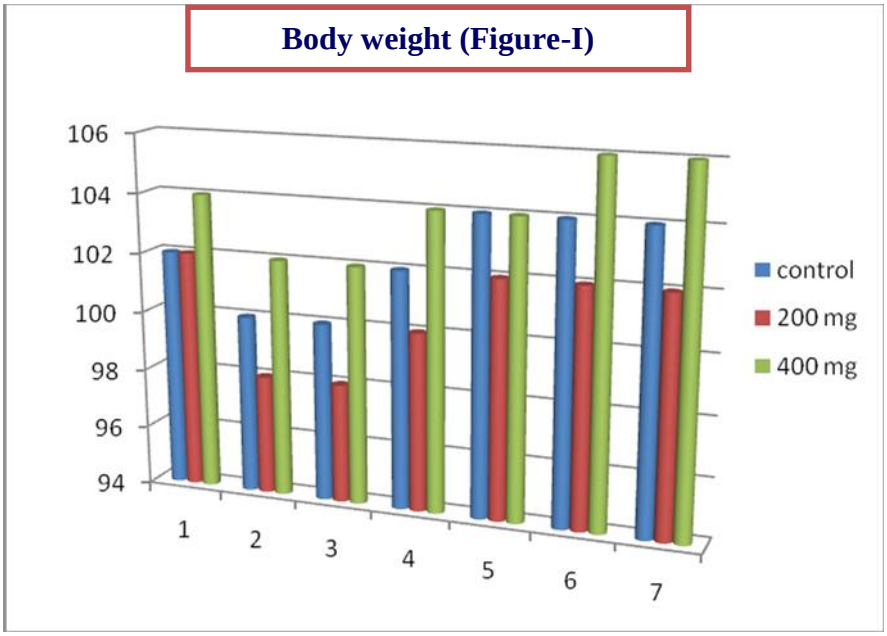
The trail drug SilasathuParpam was administered to Wister albino rats at the dose of 3200 mg/100gm. Treated with Silasathu Parpam up to 3200mg/100gm has produced no mortality in animals. Based on OECD 423 the drug is considered to be nontoxic up to the dose of 3200mg/100gm (Table I).

Table I Shows the Results of Acute Toxicity Study of Silasathu Parpam at 24 hours

Observation	Control	**200mg**	**400mg**	**800mg**	**1600mg**	**3200mg**
I. Stimulation:						
Hyperactivity	-	-	-	-	-	-
Pyloerection	-	-	-	-	-	-
Twitching	-	-	-	-	-	-
Rigidity	-	-	-	-	-	-
Irritability	-	-	-	-	-	-
Jumping	-	-	-	-	-	-
Clonic convulsions	-	-	-	-	-	-
Tonic convulsions	-	-	-	-	-	-
II.Depression:						
Ptosis	-	-	-	-	-	-
Sedation	-	-	-	-	-	-
Sleep	-	-	-	-	-	-
Loss of Traction	-	-	-	-	-	-
Loss of pinna reflex	-	-	-	-	-	-
Ataxia	-	-	-	-	-	-
Loss of Musle tone	-	-	-	-	-	-
Analgesia	-	-	-	-	-	-
III.Autonomic effect:						
Straub tail	-	-	-	-	-	-
Laboured respiration	-	-	-	-	-	-
Cyanosis	-	-	-	-	-	-
Reddening	-	-	-	-	-	-
Abnormal secretions	-	-	-	-	-	-
IV. Mortality:						
After 24 hours	-	-	-	-	-	-

All animals from control and the entire treated dose (100 and 200 mg/100gm) groups survived throughout the dosing period of 90 days for chronic 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 90 days. (Figure-I)

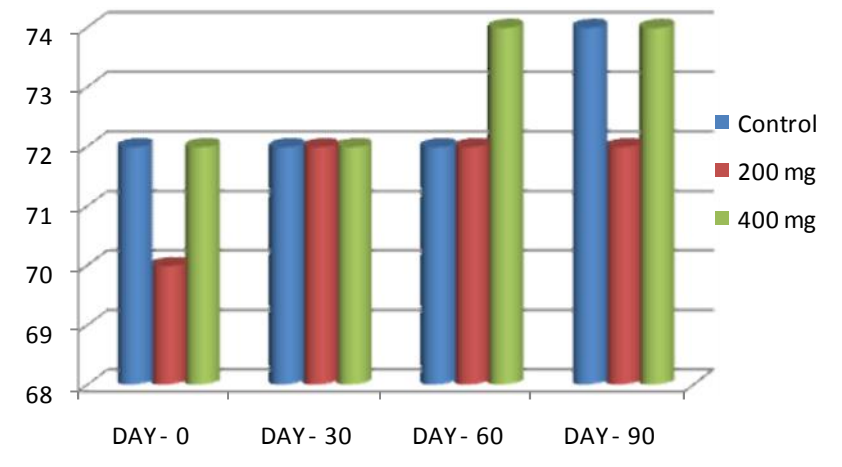
Body weight (Figure-I)

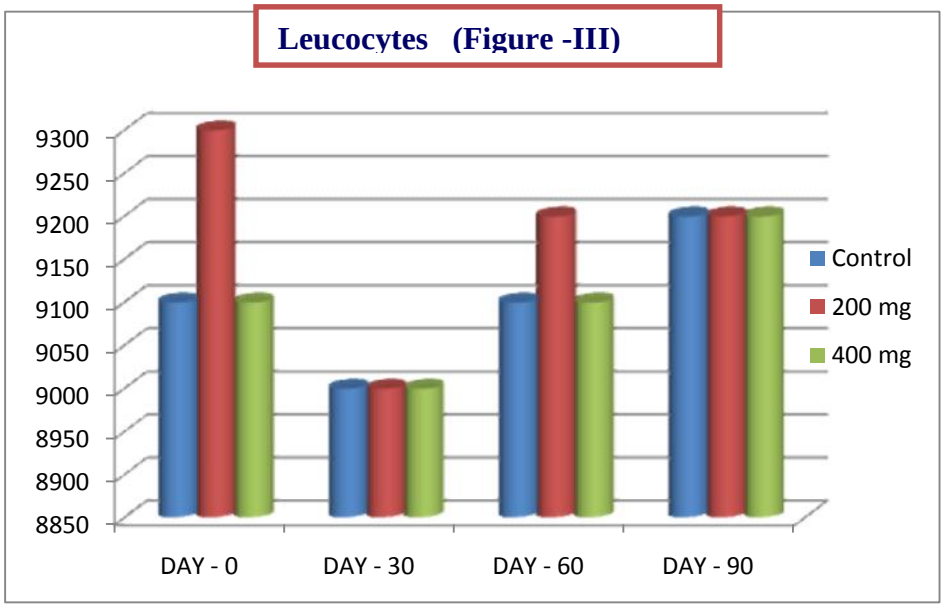


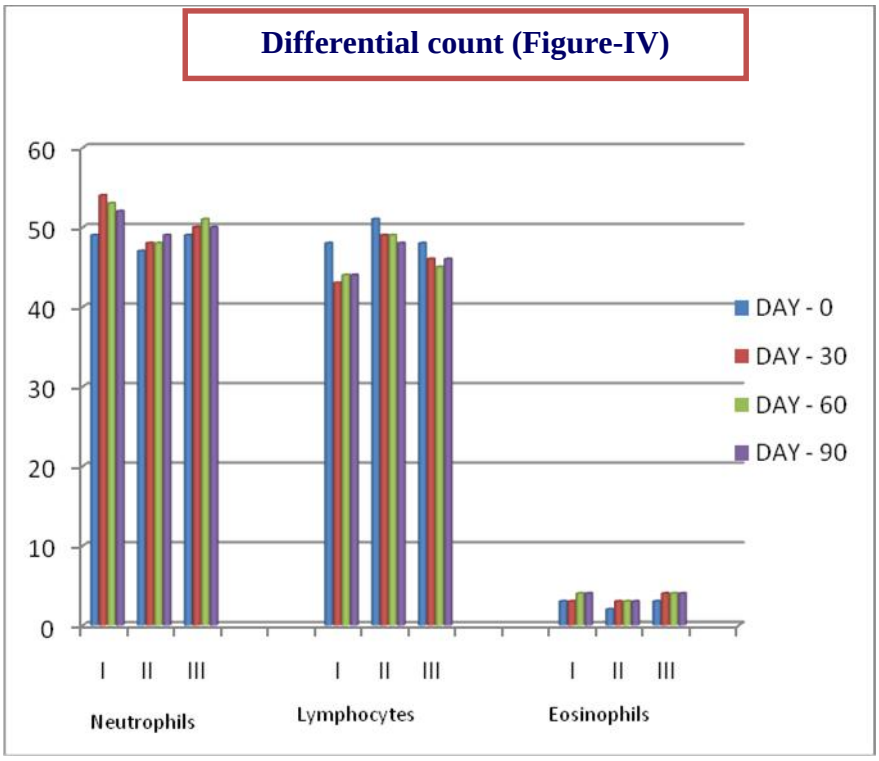
Haematological Investigations:

Blood samples of control and experimental rats was analysed for haemoglobin (Figure-II), total red blood corpuscles (RBC) (Figure-III), white blood corpuscles count (WBC) (Figure-IV). The results of haematological investigations revealed no significant changes in the values when compared with those of respective controls.

Haemoglobin (%) (Figure-II)







Histopathology:

Histopathological changes in Wister Albino Rats. (Table II)

Group I

Liver: Section shows Liver tissue normal hepatocytes and dilated Sinusoids.
Heart: section studied shows normal myocardium bundles.

Kidney: section of kidney shows normal glomeruli with normal tubules.
Brain: section shows Cerebrum with no remarkable changes.

Group II

Liver: Section shows Liver tissue normal hepatocytes and dilated Sinusoids.

Heart: section studied shows cardiac myocytes with focal atrophy.

Kidney: section of kidney shows normal glomeruli with normal tubules.

Brain: section shows cerebrum with minute foci of congestion.

Group III

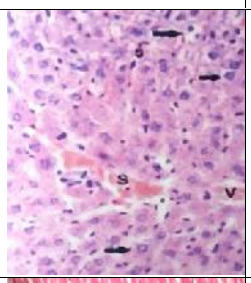
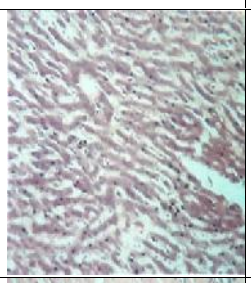
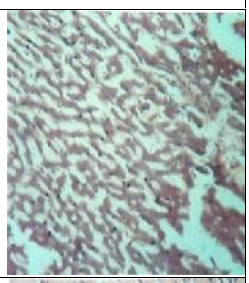
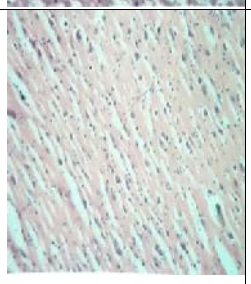
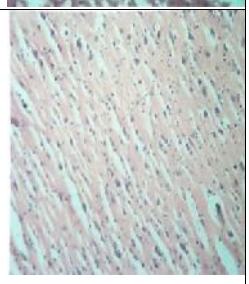
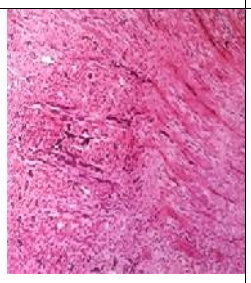
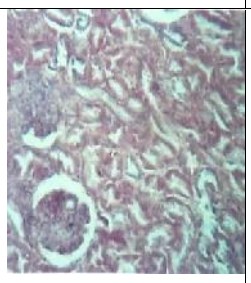
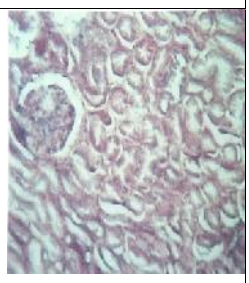
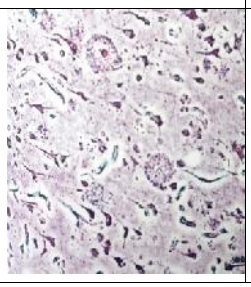
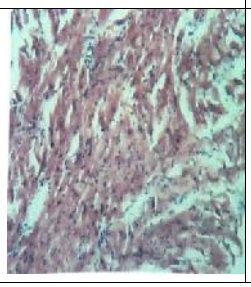
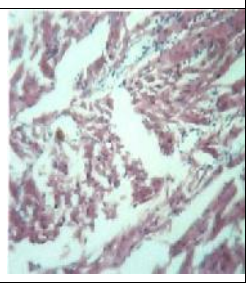
Liver: Section shows Liver tissue normal hepatocytes and dilated Sinusoids.

Heart: section studied shows mild atrophy of the cardiac myocytes with interstitial inflammation.

Kidney: section of kidney shows normal glomeruli with interstitial oedema.

Brain: section shows Cerebrum with foci of congestion.

Table – II Shows Histopathology changes in Wister Albino Rats for control, 200mg/100gm, 400mg/100gm

Organ	Control	200mg/100gm	400mg/100gm
Liver			
Heart			
Kidney			
Brain			

Statistical aspects:

There was no mortality of the animals in both acute and chronic toxicity studies, lethal dose LD_{50} of the drug could not be calculated. From bio statistical measures of SP was found to be safe upto the dose level of 3200 mg/100gm body weight of the animal.

Discussion

The preclinical toxicity study of Silasathu Parpam was conducted with the prime objective to find out whether the drug has possess any side effects or adverse reactions on long term administration.

In acute toxicity study all the animals were active and did not showed any signs of toxicity. The motor activities were normal in all the 6 groups of animals. This acute toxicity study results reveal that Silasathu parpam was nontoxic upto a dose level of 3200mg/100gm body weight of the animal.

Doses for chronic toxicity study were selected on the basis of acute toxicity study. The selected doses were 200mg/100gm and 400mg/100gm body weight of the animal.

In chronic toxicity study no signs of toxicity were observed. No changes in the haematological parameters. There was a slight increase in food and water intake which was of not much significant. No mortality occurred till the last day of the study.

Necropsy study of the major organs liver, kidney, brain and heart showed no apparent change in colour. The texture of the organs maintained and the specimens were normal on macroscopically examination when compared with that of the control group.

Histopathological examination of liver showed that the hepatocytes were normal. The microscopic view of kidney showed well maintained normal glomeruli with normal tubules, while a mild focal atrophy was noted in the heart. In group III the histological picture of liver was normal, the glomeruli remained intact and unaffected but a slight oedema of interstitium was noted. Foci of congestion in brain and a mild inflammatory change were noted in the myocardium. These changes might have produced as a result of the higher dosage. The doses selected for toxicity studies were relatively higher when compared to the clinical dose. Such a high dosage was selected to know the target organ of toxicity, to gain information related to mode of toxic action if any. This would aid in the diagnosis and treatment of toxic reactions if any such reactions occur.

Since, there was no mortality in both acute and chronic toxicity studies the lethal dose of the drug could not be calculated. The biostatistical analysis reveals that Silasathu Parpam is safe up to a dose level of 3200mg /100gm body weight of the animal.

Conclusion

Silasathu Parpam had not produced any mortality or signs of toxicity even up to a dose level of 3200mg/100gm body weight in acute toxicity study. The drug possesses very low toxicity on long term administration. This toxicity might have occurred because of the high dosage on long term administration. Since it is the right dose that differentiates a poison and a remedy, attention has to be paid in deciding the dose. The doses selected for toxicity studies were relatively higher when compared to the clinical dose. This study reveals that the therapeutic dose for silasathu parpam mentioned in the text could be concluded safe for clinical practice.

References

Sarabendhirar soolai, moola, kusta, pitha roga sikitchai muraigal Pg.No: 174.

Pulipaani jaalathiratu Pg-33

Gunapadam thathu jeeva vagupu Pg- 370, 397, 529

Anuboga vaidhya navaneetham part-3 Pg-83

Toxicology the science of poison Aavishkar publishers Jaipur – S.C.Dwivedi Nalini, Dwivedi

Modi's Medical Jurisprudence & Toxicology – Dr C.A Franklin MD

Access this Article in Online

Website: www.darshanpublishers.com

Subject: Siddha Medicine

Quick Response Code

How to cite this article:
Vikesh. B, Radha. S, Thanikaiselvi. S, Thiruthani. M. (2018). Acute and chronic toxicity study of Silasathu parpam on Wister albino rats. Int. J. Curr. Res. Biol. Med. 3(5): 6-13.
DOI: http://dx.doi.org/10.22192/ijcrbm.2018.03.05.002