Acute and Sub Acute Toxicity Studies of *Pavala Parpam*

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Abstract

Pavala parpam has long been used as a traditional medicine. In this study, the acute and sub-acute toxicity studies on Pavala parpam were performed in Albino Wistar rats. Limit test (2000mg/kg) was performed to evaluate acute toxicity of Pavala parpam. Sub-acute 28 days toxicity study was carried out at doses 100, 200 and 400 mg/kg. No abnormalities were observed during the 14 days period of acute toxicity study. The biochemical, haematological and physical parameters evaluated during the sub acute toxicity study remained slightly altered compared to the control and histopathological studies revels that liver showed slight inflammatory infiltration, brain showed slight edema, lung shows distend alveoli and congested alveolar wall, kidney showed slight lymphocytic infiltration and pancreas shows atrophic and small congested islets. Hence, the study provides evidence that Pavala parpam is not toxic on acute toxicity and sub acute toxicity studies.

Key words: *Siddha, Acute Toxicity, Pavala Parpam, Sub-Acute Toxicity*

1. INTRODUCTION

The fundamental subjects of Siddha are Vadham (Alchemy), Vaithiyam (Medicine), Yogam (Yoga), Gnanam or Thathuvam (Philosophy). As per Siddha MateriaMedica, every drug is made up of five Boothas and has got the following properties such as Suvai (Taste), Gunam (Character), Veeriyam (Potency), Pirivu (Biotransformation) and Magimai (Special Property)[1]. These drugs can be obtained for medicinal purposes from natural sources viz., 1. Mooligai (Herbal origin) 2. Thathu (Mineral origin) 3. Jeevam (Zoological origin)[2]. Siddha drugs are classified as internal and external medicines. Each type consists of 32 forms [2]. Among the 32 internal medicinal forms, *Parpams* are inorganic preparations produced by the process of Pudam (process of burning using dung cakes), burning, frying, blowing and grinding the Ulogams (Metals), Uparasams (Secondary minerals), Padanams (Arsenic compounds) with juices, Ceyaneer (pungent liquid) etc., which convert them into white ash powder [2]. Various *Parpams* such as *Sangu parpam, Muthuchippi parpam, Palagarai parpam, Aga parpam, Thanga parpam, Pavala parpam* etc., are under practice since long time [3]. These *Parpams* claimed some advantages such as deep penetration, rapid action, efficacy in minute quantities, long shelf period, no adverse interaction with herbal drugs, usefulness in obstinate and incurable diseases, wide spectrum of therapeutic indications and rejuvenating action, lack of adverse effects if properly made.

Pavala parpam is a traditional Siddha medicinal preparation and it is a well-known potent preparation having shelf life of 100 years. This marine sourced medicine is

synthesized by calcination of corals as narrated in the classical siddha literature.

The qualitative and quantitative analyses of *Pavala parpam* show that it has the contents calcium, ferrous iron, tannin and tannic acid. Various procedures for the preparation of this *Pavala parpam* are mentioned in Siddha Materia Medica such as Theran yemaga method, processing with latex of *Erukku* (*Calotropis gigantea*), leaf poultice of *Ilanthai* (*Zizyphus jujuba*) or *Thaivelai* (*Gynandropsis pentaphylla*) or *Keezhanelli* (*Phyllanthus niruri*) or Rabbit's blood or honey or sugarcane candies. They vary with adjuvant, but was found to possess diuretic, laxative, astringent, nervine tonic, spermatogenesis properties.

Pavalla parmam is also used for the treatment of Azhal aggravated diseases, excessive phlegm, eye diseases. In-vitro studies showed that *Pavala parpam* has good antimicrobial activity against the bacterial strains such as *S.mutans, S.aureus, E.coli, K.pneumoniae and P.aeruginosa.* In Anuboga Vaidhya Avaneetham, *Pavala parpam* was attributed as Sanjeevi drug particularly in the management of bleeding from the organs due to its hemostatic action [2].

Administration of drugs or chemical substance in a biological system, can lead to different types of interactions.

The types of toxicity tests which are performed in the investigation of a new drug involve acute and subacute toxicity [4]. Acute toxicity is involved in the estimation of LD_{50}, the dose which proves to be lethal to 50% of tested group of animals. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of any drug [5].

Sub acute toxicity testing is essential when the drug is meant to be administered for a long term [6]. Since detailed toxicity studieson *Pavala parpam* are not reported, the present study was undertaken is to evaluate acute and sub-acute toxicity studies on *Pavala parpam*

Objective

The objective of the present study was to conduct acute and sub-acute toxicity studies on *Pavala parpam.*

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

Pavala parpam was collected commercially from Annai Aravindh Herbals, Chennai, Tamil Nadu, during April 2015, in powdered form and retained in our laboratory for further studies. The drug was suspended in a 2% w/w CMC for the toxicity studies.

2.2 Animals

Albino Wistar rats weighing between 150-200 g were bred in the animal house of faculty of pharmacy. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 h light and dark cycle at 28°C±2°C in a well-ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 15 days prior to the commencement of the experiment. Paddy husk was used as bedding material. The animals were fed with standard pelleted diet. All animal experiments were performed with prior approval from the Institutional Animal Ethics Committee (IAEC) of the institution. IAEC No: MSRCP/SP-53/2014.

2.3 Acute Toxicity Study

Females Wistar Albino rats were used for the limit test as per OECD guide line 423. Five animals were dosed at 2000 mg/kg p.o. After dosing animals were kept separately in cages for 24 h for observation of any kind of toxic symptoms[7]. They were under close monitoring for the following 48 h and subsequently up to 14 days for better data generation.

2.4 Sub-Acute Toxicity Studies

Sub-acute toxicity studies on *Pavala parpam* was carried out as per OECD guidelines 407 with certain modifications[8]. The residue suspended in CMC was administered p.o. at varying doses (100, 200 and 400 mg/kg) for 28 days. The control group received vehicle for 28 days. Each group contained five rats.

Body Weight Measurement

The body weight of each rat was measured using a sensitive balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and on the day of sacrificing

Mortality and Clinical Signs

All the animals were observed daily for clinical signs and mortality patterns before and immediately after dosing and during the 28 days dosing period.

Organ Weight

On the 28th day, the animals were fasted overnight and on the 29th day, they were sacrificed and different organs namely the liver, brain, kidneys, lung and pancreas were carefully dissected out and their wet weight was taken and expressed in g/100 g body weight[9].

Hematological Assay [10]

On the 29th day, before the animals were sacrificed, blood samples were collected into heparinized tubes from retro orbital plexus under light ether anaesthesia for the estimation of hematological parameters such as hemoglobin concentration, RBC, WBC, ESR and clotting time.

Biochemical Estimations

Blood was collected through retro-orbital path into non heparinized tubes and centrifuged for 10 min. at 8000 rpm. The serum supernatant was collected and then used for the determination of biochemical parameters such as glucose, total protein, albumin, creatinine and SGOT[11-16].

Histopathology Study

On the 29th day animals were sacrificed and organs such as pancreas, brain, kidney, lungs and liver, pancreas were isolated and subjected to histopathological analysis.

2.5. Statistical Analysis

The results were reported as mean ± SEM of n=6 in each group. Total variations, present in a set of data were estimated by one way ANOVA followed by Tukey-kramer multiple comparison test

3. RESULTS AND DISCUSSION

The results of acute toxicity study showed that Pavala parpam was safe up to 2000 mg/kg, b.w, p.o. in Albino Wistar rats. In sub-acute toxicity study no significant changes were observed in body weight of animals in the treatment groups compared with the control group. No mortality was observed during the study. Food and water intake had been normal throughout the treatment period (Table 1).

Tukey Krammer Multiple Comparison test results were non-significant when compared to the control; no significant changes were observed in body weight on sub-acute administration of Pavala parpam for 28 days. Slight changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), hematocrit value and blood clotting time in the treated groups compared to the control group. The mean values these parameters are listed in (Table 2).

Tukey Krammer Multiple Comparison test results were non-significant when compared to the control; slight changes wereobserved in the hematological parameters on sub-acute administration of Pavala parpam for 28 days.

Table 1. Composition of the films

Group	1st day	1st week	2nd week	3rd week	4th week
Control	189 ± 20.09	208 ± 18.89	204 ± 18.29	208 ± 18.44	210 ± 18.55
100 mg/kg	196 ± 1.0	218.16±6.04	220 ± 5.79	215 ± 8.81	220.6±7.546
200 mg/kg	188 ± 6.38	209.50±5.46	213 ± 0.89	211 ± 7.42	225 ± 6.54
400 mg/kg	184 ± 10.16	206.80±8.32	210 ± 10.4	217 ± 10.71	219 ± 10.21

Values are expressed in mean ± SEM, n = 6.

Table 2. Haematological parameters

Group	RBC 10^6 mil/mm^3	WBC 10^3 mil/mm^3	Hematocrit %	Hb g/%	Clotting time sec
Normal control	8.22±1.60	6.12±1.10	94.50±1.75	14.80±0.50	102.40±2.50
100 mg/kg	10.12±1.30	7.22±0.50	98.12±0.40	13.80±1.32	124.50±3.50
200 mg/kg	11.24±0.56	8.8±1.07	96.20±1.10	13.81±.38	131.34±4.25
400 mg/kg	11.98±1.45	10.84±0.45	88.32±1.70	12.80±0.35	130.20±2.40

Table 3. Biochemical parameters

Group	SGOT IU/L	Total protein g/l	Albumin g/l	Glucose mg/dl	Creatinine mg/dl
Normal control	25.20±4.57	6.43±1.10	2.80±1.20	93.60±0.60	3.20±2.20
100 mg/kg	30.15±5.75	6.73±1.15	3.11±0.50	97.44±1.50	3.10±1.35
200 mg/kg	36.23±6.55	6.80±0.55	3.43±0.60	90.11±0.35	3.20±0.50
400 mg/kg	35.45±3.30	6.80±1.25	3.88±1.25	97.80±2.10	3.50±1.10

Values are expressed in mean ± SEM, n = 6

Slight changes were observed in serum total protein, albumin, glucose, SGOT and creatinine treated groups compared with the control group (Table 3).

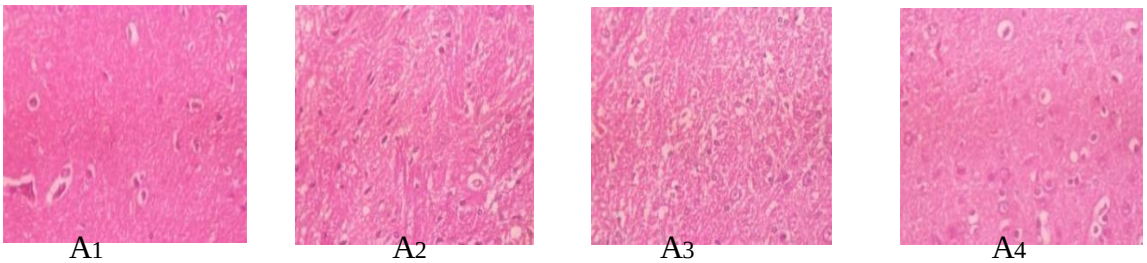
Tukey Krammer Multiple Comparison test results were non-significant when compared to the control; slight changes were observed in biochemical parameters on sub-acute administration of *Pavala parpam* for 28 days. All the animals were sacrificed on the 29th day after completion of the study and wet weight of major organs like heart, brain, kidney, liver and spleen were taken. Results are shown in (Table 4).

Table 4. Wet weight of the organ (g/100 g of animal weight)

Group	Pancreas	Brain	Liver	Kidney	Lung
Normal control	0.50±0.08	0.89±0.05	3.80±1.20	0.70±0.05	0.50±1.05
100 mg/kg	0.53±0.04	0.90±0.04	3.50±1.10	0.75±0.10	0.55±0.08
200 mg/kg	0.53±0.05	0.92±0.08	3.60±0.75	0.72±0.08	0.53±0.08
400 mg/kg	0.55±0.05	0.95±0.05	3.70±1.05	0.70±0.10	0.54±0.06

Values are expressed in mean ± SEM, n = 6

Tukey Krammer Multiple Comparison test results were non-significant when compared to the control; no significant changes were observed in the organ weights on sub-acute administration of Pavala *Parmap* for 28days. Histopathology studies on brain, kidney, lungs, liver and pancreas showed that liver showed slight inflammatory infiltration, brain showed slight edema, lung shows distend alveoli and congested alveolar wall, kidney showed slight lymphocytic infiltration and pancreas shows atrophic and small congested islets in treated groups compared to control. The photomicrographs of organs treated with *Pavala parpam* and control (Fig 1).



A1 A2 A3 A4

Fig.1 A Histopathological studies of various organs in sub acute toxicity studies of *Pavala parpam* Histopathological studies of brain in sub acute toxicity studies of *Pavala parpam*

A_1: Section of brain treated with normal saline: A_2: Section of brain treated with *Pavala parpam* (100 mg/kg) A_3: Section of brain treated with *Pavala parpam* (200 mg/kg):A_4: Section of brain treated with *Pavala parpam* (400 mg/kg)

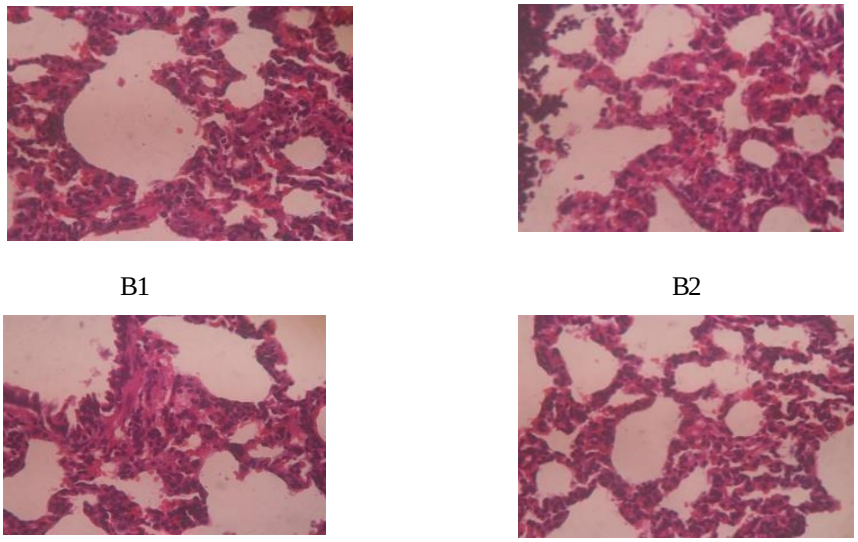


Fig. 1B Histopathological studies of lung in sub-acute toxicity studies of *Pavala parpam*

B_1: Section of lung treated with normal saline; B_2: Section of lung treated with *Pavala parpam* (100 mg/kg) B_3: Section of lung treated with *Pavala parpam* (200 mg/kg) : B_4: Section of lung treated with *Pavala parpam* (400 mg/kg).

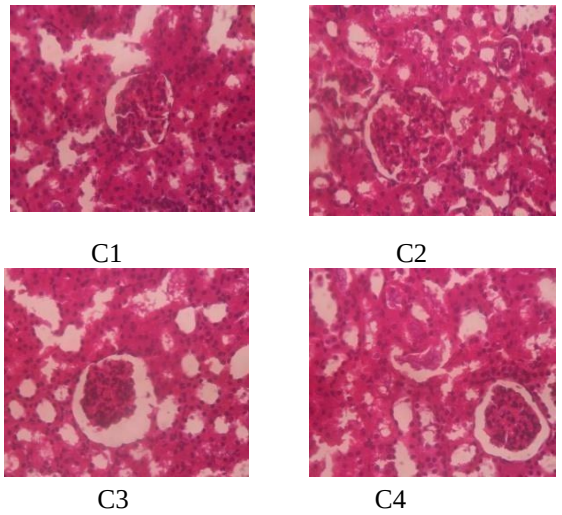


Fig. 1C Histopathological studies of kidney in sub-acute toxicity studies of *Pavala parpam*

C_1: Section of kidney treated with normal saline
C_2: Section of kidney treated with *Pavala parpam* (100 mg/kg)
C_3: Section of kidney treated with *Pavala parpam* (200 mg/kg)
C_4: Section of kidney treated with *Pavala parpam* (400 mg/kg).

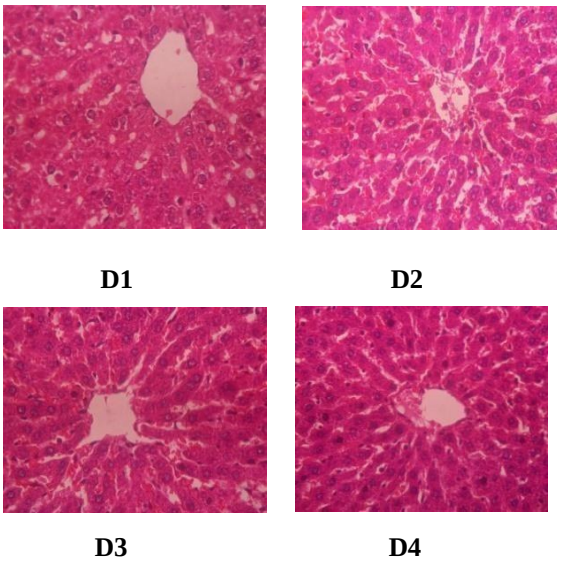


Fig. 1D Histopathological studies of liver in sub-acute toxicity studies of *Pavala parpam*

D_1: Section of liver treated with normal saline
D_2: Section of liver treated with *Pavala parpam* (100 mg/kg)
D_3: Section of liver treated with *Pavala parpam* (200 mg/kg)
D_4: Section of liver treated with *Pavala parpam* (400 mg/kg).

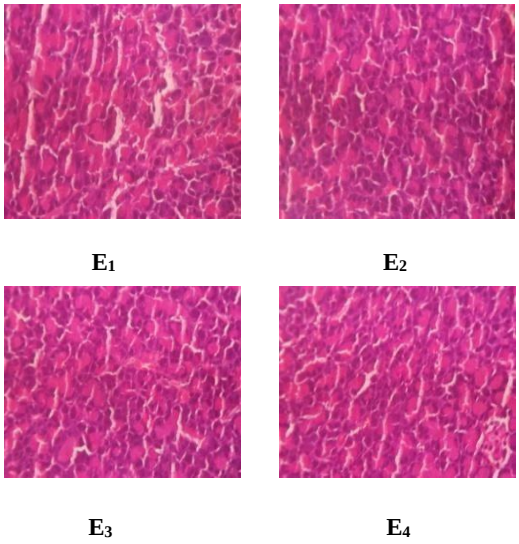


Fig. 1E Histopathological studies of pancreas in sub-acute toxicity studies of *Pavala parpam*

E_1: Section of pancreas treated with normal saline
E_2: Section of pancreas treated with *Pavala parpam* (100 mg/kg)
E_3: Section of pancreas treated with *Pavala parpam* (200 mg/kg)
E_4: Section of pancreas treated with *Pavala parpam* (400 mg/kg)

Acute toxicity study of *Pavala parpam* was performed according to OECD guide line 423. No mortality and abnormalities were observed at limit test dose of 2000 mg/kg, hence, considered safe up to 2000 mg/kg. Based on the result of acute toxicity study, sub-acute toxicity studies were carried out with doses 100, 200 and 400 mg/kg. *Pavala parpam* was administered orally as recommended by OECD guideline 407. All the animals were free of intoxicating signs and physical changes throughout the dosing period of 28 days. No significant change in body weight was observed during the treatment period. There was slightly rise in RBC count, WBC count, haematocrit, haemoglobin levels and clotting time in the treatment groups compared to the control group. The possibility of stimulation of erythropoiesis and leukocytosis by the extract needs to be explored further.
Examination of biochemical parameters in serum showed slight changes in serum total protein, albumin, glucose, SGOT and creatinine treated groups compared with the control group. Wet weight of the major organs like lungs, brain, kidney, liver and pancreas was taken and showed no significant changes in weight of the major organs in treated group when compare with control.
Histopathology studies on some of the major orangs like brain, kidney, lungs, liver and pancreas was carried out and showed that liver showed slight inflammatory infiltration, brain showed slight edema, lung shows distend alveoli and congested alveolar wall, kidney showed slight lymphocytic infiltration and pancreas shows atrophic and small congested islets intreated groups compared to control.

4. CONCLUSION

No mortality and abnormalities were observed at limit test dose of 2000 mg/kg in acute toxicity study, hence, considered safe up to 2000 mg/kg.
No significant change in body weight, slightly rise in RBC count, WBC count, hematocrit, hemoglobin levels and clotting time was observed.
Serum total protein, albumin, glucose, SGOT and creatinine showed slight changes. No significant changes was observed in histopathological studies of Lungs, Brain, Kidney, Liver and Pancreas.
Hence, this study provide evidence that *Pavala parpam* is not toxic in acute and sub-acute administration

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REFERENCES

1. Uthamarayan KS. Aymboodha paguppu thanmai. In: Thotrakirama Araichium Siddha Maruthuva Varalarum. 6th reprint. Chennai. Dept of Indian Medicine and Homeopathy. 2010; 1: 342.
2. Thiyagarajan R. Introduction, Apparatus, Drugs, Nine gem stones. In: AnaivariR Anandan, Thulasimani M. Siddha Materia Medica (Mineral & Animal section). 1st ed. Chennai. Dept ofIndian Medicine and Homeopathy. 2008;1: 373-374.
3. Uthamarayan KS, Thiyagarajan R, Ramanan MV. In: Bharathathin Siddha Marunthugal (Seimurai kurippu nool) Part I. 1st ed. New Delhi: Controller of Publication, Health department - Delhi, Ministry for Health and Family welfare, Govt of India. 1984: 31-36.
4. Robinson S, Ockert D, Stei P. Challenging the regulatory requirement for conventional toxicity studies in pharmaceutical drug. Drug Dev Toxicol 2007; 3:96-104.
5. Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose. Curr Sci 2007; 93: 917-20.
6. Steve O, Ogbonnia, Florence E. Evaluation of acute and sub chronic toxicity of *Stachytarpheta angustifolia* extract in animals. African J Biotech 2009; 8(9):1793-99.
7. Organization for Economic Co-operation and Development (OECD) Guideline No. 423. Acute oral toxicity in animals. OECD/OCDE No. 423, adopted 17th Dec, 2001. Available from: http://www.ntp.niehs.nih.gov / iccvam /suppdocs/feddocs/oecd gl 423.
   Organization for Economic Co-operation and Development (OECD) Guideline No. 407. Repeated Dose 90-day Oral Toxicity Study in Rodents. OECD/OCDE No. 407, Jul 27; 1995. 407. Adopt 3rd Oct 2008. Available from http: // www. Oecd-library.org /environment / test-no -407 -repeated-dose-28-day-oral-toxicity – study - in-rodents_
8. Payasi A, Chaudhury M, Sing BM, Gupta A. Sub-acute toxicity studies of Paracetamol infusion in Wistar albino rats. Int J Pharm Sci Drug Res 2010; 2(2): 142-45.
9. Ghai CL. A Text Book of Practical Physiology. 5th Ed. New Delhi: Jaypee Brothers Medical Publishers. 2003:1; 19-21.
10. Trinder P. Estimation of serumglucose. Annals. Clin.Bio Chem 1969; 6:24.
11. Weichselbaum TE. Total Serum Protein estimation. Am J Clin Path 1946; 16: 40.
12. Doumas BT. Estimation of Serum Albumin. Clin Chem Acta 1971; 3(1): 87.
13. Chaney AL, Marbach EP. Estimation of Serum Urea. Clin Chem. 1962:8; 130.
14. Browers LD. Estimation of Creatinine in fluid sample. Clin Chem 1980; 26: 551.
15. Bergmeyer HU, Herder M, Rej R. Estimation of Aspartate transaminase. Clin Chem Biochem 1986; 24: 49-54.