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 reveals 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 Materia Medica, every drug is made up of five Boothas and has got the following properties such as Suvai (Taste), Gunam (Character), Veeriyan (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, Muthuchipparpam, 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 Erukk (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 parpam 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. 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. Acute toxicity is involved in the estimation of LD₅₀, 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.

Sub acute toxicity testing is essential when the drug is meant to be administered for a long term. Since detailed toxicity studies on Pavala parpam are not reported, the present study was undertaken 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 Anmai 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. 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. 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.

**Hematological Assay**

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, hematocrit value and blood 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.

**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 were observed in the hematological parameters on sub-acute administration of Pavala parpam for 28 days.
Table 1. Composition of the films

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

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

Table 2. Haematological parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC 10^6 mil/mm³</th>
<th>WBC 10^3 mil/mm³</th>
<th>Hematocrit %</th>
<th>Hb g/%</th>
<th>Clotting time sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8.22±1.60</td>
<td>6.12±1.10</td>
<td>94.50±1.75</td>
<td>14.80±0.50</td>
<td>102.40±2.50</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>10.12±1.30</td>
<td>7.22±0.50</td>
<td>98.12±0.40</td>
<td>13.80±1.32</td>
<td>124.50±3.50</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>11.24±0.56</td>
<td>8.8±1.07</td>
<td>96.20±1.10</td>
<td>13.81±3.8</td>
<td>131.34±4.25</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>11.98±1.45</td>
<td>10.84±0.45</td>
<td>88.32±1.70</td>
<td>12.80±0.35</td>
<td>130.20±2.40</td>
</tr>
</tbody>
</table>

Table 3. Biochemical parameters

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

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)

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

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 Parpam for 28 days. 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](image1.png) ![A2](image2.png) ![A3](image3.png) ![A4](image4.png)

**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*

A1: Section of brain treated with normal saline; A2: Section of brain treated with *Pavala parpam* (100 mg/kg) A3: Section of brain treated with *Pavala parpam* (200 mg/kg); A4: Section of brain treated with *Pavala parpam* (400 mg/kg)
Fig. 1B Histopathological studies of lung in sub-acute toxicity studies of *Pavala parpam*

B1: Section of lung treated with normal saline; B2: Section of lung treated with *Pavala parpam* (100 mg/kg)
B3: Section of lung treated with *Pavala parpam* (200 mg/kg); B4: Section of lung treated with *Pavala parpam* (400 mg/kg).

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

C1: Section of kidney treated with normal saline
C2: Section of kidney treated with *Pavala parpam* (100 mg/kg)
C3: Section of kidney treated with *Pavala parpam* (200 mg/kg)
C4: Section of kidney treated with *Pavala parpam* (400 mg/kg).

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

D1: Section of liver treated with normal saline
D2: Section of liver treated with *Pavala parpam* (100 mg/kg)
D3: Section of liver treated with *Pavala parpam* (200 mg/kg)
D4: Section of liver treated with *Pavala parpam* (400 mg/kg).
Fig. 1E Histopathological studies of pancreas in sub-acute toxicity studies of Pavala parpam

E1: Section of pancreas treated with normal saline
E2: Section of pancreas treated with Pavala parpam (100 mg/kg)
E3: Section of pancreas treated with Pavala parpam (200 mg/kg)
E4: 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 organs 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 in treated 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


