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TOXICOLOGICAL EVALUATION OF SIDDHA FORMULATION PAVAZHA SILASATHU PARPAM IN WISTAR RATS

M. Maha Sundari@ Sudha [*1],L.Parwin[2], U.Chitra[3],N.Anbu[4]

[*1,2] *P.G Scholar, Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India*
[3] *Lecturer, Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India*
[4] *Head, Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India*

ABSTRACT

Traditional medicine has become a popular form of healthcare; even though several differences exist between traditional and conventional pharmacological treatments, Indigenous medicine needs to be tested for efficacy using conventional trial methodology and several specific herbal extracts have been demonstrated to be efficacious for specific conditions. Nevertheless the public is often mislead to believe that all natural treatments are inherently safe, so research in this area must be intensified. Siddha drug pavazha silasathu parpam (PSP) is a herbo mineral preparation indicated for therapeutic management of cystitis, but still now there is no documentary evidence claiming the safety profile of this formulation hence the main aim of the present study is to screen the safety profile of PSP in selective rodent model. In the acute study, a single dose of 2000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses (200 and 400 mg/kg/day) of the test drug PSP were administered for 28 days and the followed by which biochemical and hematological parameters were evaluated. Results of acute toxicity study revealed that the drug PSP was found to be non-toxic at a dose of 2000 mg/kg b.w. In the sub-acute toxicity study there is no significant variations in body weight, hematological and biochemical parameters were observed in the experimental groups at the dose of 200 and 400 mg/kg with no mortality in both male and female rats subjected to the study. In conclusion the results of the acute and sub-acute toxicity study clearly demonstrate that the formulation PSP was safe and long-term administration of drug may not claim any adverse event.

KEY WORDS: ***Siddha drug, Pavazha silasathu parpam, Safety, Acute, Sub-acute, Hematological, Biochemical parameters***

Corresponding Author: M. Maha Sundari@ Sudha, P.G Scholar, Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India

1. Introduction

As the global use of herbal and herbo-mineral products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult [1]. It is also common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information [2]. It has become essential, therefore, to furnish the general public including healthcare professionals with adequate information to facilitate better understanding of the risks associated with the use of these products and to ensure that all medicines are safe and of suitable quality.

The lack of definite and complete information about the traditional medicine and its derivatives. Herbal derived remedies need a powerful and deep assessment of their pharmacological qualities and safety that actually can be realized by new biologic technologies like pharmacogenomic, metabolomics and microarray methodology [3]. Because of the large and growing use of natural derived substances in all over the world, it is not wise to rely also on the tradition or supposed millenarian beliefs; explanatory and pragmatic studies are useful and should be considered complementary in the acquisition of reliable data both for health caregiver and patients [4]. Traditional therapy is a holistic therapy, integrating emotional, mental and spiritual levels. Life style, emotional, mental and spiritual considerations are part of any naturopathic approach. The use of herbs does not generally involve “drug” actions or adverse effects. Of course, informed knowledge of the effects of medicinal plants and minerals as well as doing a clinical trial to understand the appropriate medical application is necessary. It has been suggested that we use the terms indications and contraindications for using an herb instead of “side effects” [5-8].

Siddha practice emerged from the Indian southern zone, spectacularly ameliorates the diseases and disorders that occurs in the humans due to several life style factors. It becomes the moral responsibility of the siddha practitioners to restore the basic knowledge of siddha drugs and its relative effects among the general public. Siddha drug pavazha silasathu parpam (PSP) is a herbo mineral preparation indicated for therapeutic management of cystitis, but still now there is no documentary evidence claiming the safety profile of this formulation hence the main aim of the present study is to screen the safety profile of PSP in selective rodent model

2. Materials and Methods

2.1. Animal

Healthy adult wistar albino rats were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air supported by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬± 2˚C and relative humidity 50–65%. They were provided with standard pelleted feed and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of Science and technology, Chennai, Tamil Nadu, India with the IAEC approval number: SU/CLATR/IAEC/XV/168/2020

2.2. Acute toxicity Study

The animals were fasted overnight (08- 12 hrs) with free access to water. Study was conducted with single oral administration of study drug Pavazha Silasathu parpam (PSP) at the dose of 2000mg/kg (p.o) to experimental rats. The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S, C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation,

diarrhea, lethargy, sleep, coma and mortality were observed with special attention [9]. Body weight was recorded periodically. At the end of the experiment all animals were subjected to gross necropsy and observed for pathological changes.

2.3. Sub-Acute toxicity Study

Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the start of treatment. The female rats used for the study were nulliparous and non-pregnant. The animals were randomly divided into control group and drug treated (low and high dose) with equal ration of male and female rats were selected and divided into three groups. Each group consist of 06 animals (03 males and 03 females). First group served as a control and other two groups were treated with test drug PSP (200 and 400 mg/kg/day) for 28 days.

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28th day, the animals were fasted for overnight with free access to water. On 29th day the animals were sacrificed with excess dose of anesthesia as listed in the CPCSEA annexure. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra acetate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs were harvested and carefully examined for gross lesion, histological assessment and interpretation [10].

2.4. Hematological analysis

Blood samples were analyzed using established procedures with the aid of automated mindray hematology analyzer 2800. Parameters evaluated includes Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

2.5. Biochemical analysis [11]

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very low density Lipoprotein (VLDL), Triglycerides (TGL), Total Cholesterol, Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using mind ray auto analyzer model BS 120.

2.6. Histopathological evaluation [12]

Vital organs were harvested and the histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic analysis. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.7. Statistical analysis [13]

The statistical analysis will be carried by one-way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

3.Results

3.1. Assessment of clinical signs in rats treated with PSP on Acute toxicity study

The dose of PSP used for acute toxicity study is 2000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

3.2. Quantitative data on the body weight of rats treated with PSP in Acute toxicity study

No significant change was observed in body weight of female rats treated with PSP at the dose of 2000mg/ kg. The results were tabulated in Table 2.

3.3. Fecal Pellet consistency analysis of rats treated with PSP in acute and sub-Acute toxicity study

Rats of control and treatment group were allowed to explore to open field on clean and sterile Stainless steel tray. The collected pellets were analysed for consistency, color, Shape, Presence of blood cells etc. The results were tabulated in Table 3.

3.4. Assessment of clinical signs in rats treated with PSP on Sub-Acute toxicity study

The dose of PSP used for sub-acute toxicity study is 200 and 400 mg/kg. No mortality observed at this dose level, further no significant change with respect to clinical signs on sub-acute toxicity observed for the

period of 28 days. The results were tabulated in Table 4.

3.5. Effect of PSP on Body weight of Rats in Sub-acute toxicity study

No significant change was observed in body weight of both male and female rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 5.

3.6. Quantitative data on the food and water intake of rats treated with PSP for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 6.

3.7. Effect of PSP on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 7.

3.8. Effect of PSP on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 8.

3.9.Effect of PSP on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 9.

3.10.Effect of PSP on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 10.

3.11. Quantitative data on absolute Organ weight of male rats belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of male rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 11.

3.12. Quantitative data on absolute Organ weight of female rats belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of male rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 12.

3.13. Effect of PSP on Histopathological changes of Male rat in Sub-acute oral toxicity study

Microscopic observation of vital organs belongs to male rats presenting the following architecture as shown in figure 1.

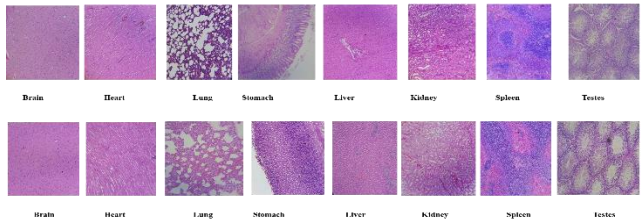

Figure 1: Histopathology of Male belongs to control and high dose treated group

3.14. Effect of PSP on Histopathological changes of Female rat in Sub-acute oral toxicity study

Microscopic observation of vital organs belongs to female rats presenting the following architecture as shown in figure 2.

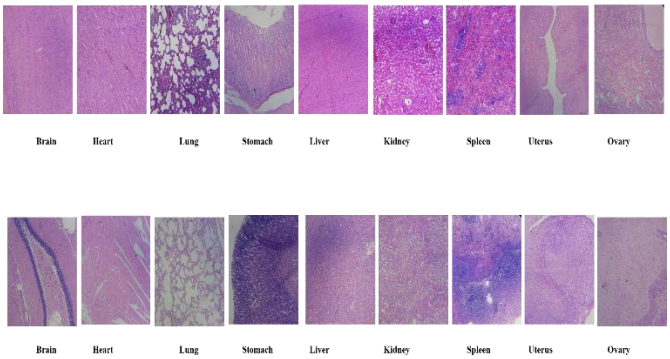

Figure 2: Histopathology of Female belongs to control and high dose treated group

4. Discussion

Traditional medicines play a major role in the rural areas in-spite of the development in synthetic and semi-synthetic drugs for the treatment of different ailments. The utility of these herbal medicines is showing a tremendous shift towards the overuse [14]. As father of toxicology, Paracelsus said "All substances are poisons; there is none which is not a poison. It is the right dose which differentiates remedy from poison" [15], it is the need of this hour is to

conduct research on the safety profile of the traditional drugs. The medicines of natural origin is expected to have very less toxicity but certain ingredients used in traditional medicines are been reported to exhibit toxic effect [16, 17].

Acute toxicity test assesses the adverse effects that occur within a short timeafter administration of a single dose of a test substance. This testing is performed principally in rodents and is usually done early in the development of a new chemical or product to provide information on its potential toxicity [18].From the results of the present study it was observed that the dose of PSP used for acute toxicity study is 2000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and for a period of 14 days. There is no significant change in the body weight, organ weight and gross observational changes of the treated animals which confirms the wide margin of safety of the study drug.

Subacute studies provide information on dosage regimens, target organ toxicity, and identify observable adverse effect that may affect the average life span of experimental animals. The body weight changes serve as a sensitive indication of general health status of animals [19]. The dose of PSP used for sub-acute toxicity study is 200 and 400 mg/kg. No mortality observed at this dose level, further no significant change with respect to clinical signs on sub-acute toxicity observed for the period of 28 days.

Analysis of haematological parameters are used to study the extent of toxicity of drug substances including plant extracts [20]. Haematopoiesis is the process of blood cell formation. Changes in the haematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies [21]. In the present investigation it was observed that there is no statistically significant differences were recorded in hematological parameters (RBC, WBC, PLT, Hb, MCH, MCV and other blood cells) of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w.

High levels of liver enzymes are signs of hepatocellular toxicity [22], whereas a decrease may indicate enzyme inhibition [23]. However, ALT is the most sensitive marker of liver damage or toxicity since AST is also found in abundance in kidneys, testes, cardiac and skeletal muscles [24]. Further results of the present study indicates no statistically significant differences in serum biochemistry parameters with reference to BUN, SGOT, SGPT, bilirubin, lipid profile of rats treated with PSP at low and high dose of 200 and 400 mg/ kg b.w

5. Conclusion

In conclusion the results of the acute and sub-acute toxicity study clearly demonstrate that the formulation pavazha silasathu parpam was safe and long-term administration of drug may not claim any adverse event. The oral LD50 of the siddha formulation pavazha silasathu parpam has been shown to be greater than 2000 mg/kg and is generally considered safe. Further the subacute toxicity study indicates that repeated intake of pavazha silasathu parpam (200 & 400 mg/kg) for 28 days did not alters the haematological, serological and histological profile of the rats belongs to treatment group. Hence, this study suggests that both low and high dose pavazha silasathu parpam was considered as safe on long term usage.

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Table 1: Clinical signs in rats on acute toxicity study

Clinical Signs Parameters for the duration of 14 days	Test Drug PSP- 2000mg/ Kg
Lacrimation	Absence
Salivation	Absence
Animal appearance	Normal
Tonic Movement	Absence
Clonic Movement	Absence
Laxative action	Absence
Touch Response	Normal
Response to Sound	Normal Response
Response to Light	Normal Response
Mobility	Normal Response
Respiratory Distress	Nil
Skin Color	Normal
Stereotype behavior	Absence
Piloerection	Absence
Limb Paralysis	Absence
Posture	Normal
Open field behavior	Normal
Giat Balancing	Normal
Freezing Behaviors	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Color	Yellowish
Urine pH	5 -7
Urine -Glucose	Absence
Urine -Ketones	Absence
Urine- Bilirubin	Absence
Urine-Blood Cells	Negative
Urine - Pus cells	Negative
Mortality	Nil

Table 2: Body weight of rats in acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	**Final Body Weight (After Treatment)**
PSP 2000 mg/kg	185.7 ± 3.327	194.3 ± 3.266

Values are mean ± S.D (n = 6 per group).

Table 3: Fecal Pellet consistency analysis of rats in acute and sub-Acute toxicity study

Acute Toxicity Study	
Analysis	**PSP**
Consistency	Soft
Shape	Oblong
Color	Pale greenish
Mucous Shedding	Absent
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Control**	**PSP 200mg/kg**	**PSP 400mg/kg**
Consistency	Rigid	Soft	Soft
Shape	Oblong	Oblong	Oblong
Color	Greenish	Pale greenish	Pale greenish
Mucous Shedding	Absence	Absence	Absence
Blood Cells	Absent	Absent	Absent
Signs of Infection	None Observed	None Observed	None Observed

Table 4: Clinical signs of rats in Sub-Acute toxicity study

Clinical Signs Parameters for the duration of 28 days	Control	PSP 200 mg/kg	PSP 400 mg/kg
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Absence	Absence
Animal appearance	Normal	Normal	Normal
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Absence	Absence
Touch Response	Normal	Normal	Normal
Response to Sound	Normal Response	Normal Response	Normal Response
Response to Light	Normal Response	Normal Response	Normal Response
Mobility	Normal Response	Normal Response	Normal Response
Resp.Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence
Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Giat Balancing	Normal	Normal	Normal
Freezing Behaviour	Absent	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed	None Observed
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	6 to 7	5 to 7	5 to 7
Urine -Glucose	Absence	Absence	Absence
Urine -Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Absence	Absence
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

Table 5: Body weight of rats in Sub-Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	Final Body Weight (After Treatment)
Control	183.7 ± 3.266	233.8 ± 15.13
PSP 200 mg/kg	187 ± 4.604	234.5 ± 13.4
PSP 400 mg/kg	186.7 ± 4.885	242.3 ± 17.75

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

Table 6: Food and water intake of rats in Sub-acute toxicity study

Dose	Average Food and Water Intake	
	Food Intake in gms	Water intake in ml
Control	14.4 ± 1.8	25 ± 2.19
PSP 200 mg/kg	14.6 ± 2.33	23.17 ± 2.31
PSP 400 mg/kg	14.67 ± 1.63	23.67 ± 2.42

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 7: Hematological parameters of rats in Sub-acute oral toxicity study

Group	RBC (×10 ^{6}µl)	WBC (×10 ^{3}µl)	PLT (×10 ^{3}µl)	HGB (g/dl)	MCH (pg)	MCV (fl)
Control	15.47 ± 23.29	7.8 ± 2.225	547 ± 188	13.73 ± 1.6	19.4 ± 4.316	60.67 ± 5.027
PSP 200 mg/kg	7.017 ± 0.6969	7.883 ± 0.5776	754.7 ± 110.4	12.05 ± 1.263	17.97 ± 3.629	61.9 ± 6.534
PSP 400 mg/kg	5.967 ± 0.3445	8.417 ± 0.9152	685.7 ± 163	13.15 ± 1.696	16.07 ± 4.389	58.82 ± 4.923

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 8: Hematological parameters of rats in Sub-acute oral toxicity study

Group	Neutrophils 10^{3} /mm ^{3}	Eosinophils (%)	Basophils (%)	Lymph (%)	Mon (%)
Control	2 ± 0.7483	1.467 ± 0.216	0.3333 ± 0.5164	73.17 ± 10.04	3.433 ± 0.3502
PSP 200 mg/kg	2.867 ± 0.5164	1.367 ± 0.3445	0 ± 0	76.1 ± 6.338	2.25 ± 1.045
PSP 400 mg/kg	2.483 ± 0.4262	1.7 ± 0.4733	0.1667 ± 0.4082	73.82 ± 8.386	2.48 ± 0.5495

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 9: Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

Group	BUN (mg/dl)	Serum Creatinine (mg/dl)	Total Bilirubin (mg/dl)	SGOT (IU/L)	SGPT (IU/L)
Control	14.67 ± 4.367	0.55 ± 0.2881	0.4667 ± 0.1862	107.5 ± 25.77	34 ± 15.05
PSP 200 mg/kg	14.17 ± 3.371	0.65 ± 0.2168	0.35 ± 0.1871	91.33 ± 44.39	36.17 ± 8.886
PSP 400 mg/kg	14.33 ± 3.559	0.65 ± 0.2881	0.4 ± 0.1414	79.5 ± 5.394	34.67 ± 17

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 10: Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

Group	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TG (mg/dl)
Control	141.2 ± 27.72	57.5 ± 9.182	67.5 ± 24.47	35.37 ± 51.88	47.5 ± 7.662
PSP 200 mg/kg	145.5 ± 21.98	67.67 ± 12.24	62.67 ± 15.63	15.12 ± 2.759	35.77 ± 18.3
PSP 400 mg/kg	130.4 ± 17.13	60.33 ± 9.309	55.83 ± 19.45	14.23 ± 2.265	40.67 ± 5.354

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

Table 11: Quantitative data on absolute Organ weight of male rats in sub-acute toxicity study

Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Testes
Control	1.7 ± 0.19	0.5567 ± 0.06658	1.15 ± 0.04359	1.357 ± 0.005773	4.783 ± 1.282	0.4867 ± 0.1274	1.377 ± 0.1106	1.387 ± 0.07371
PSP 200 mg/kg	1.777 ± 0.1387	0.4867 ± 0.07095	1.187 ± 0.05508	1.33 ± 0.1054	3.983 ± 0.4366	0.6633 ± 0.1332	1.4 ± 0.09644	1.393 ± 0.2122
PSP 400 mg/kg	1.583 ± 0.1115	0.48 ± 0.1153	1.23 ± 0.09539	1.26 ± 0.1054	4.56 ± 1.071	0.7 ± 0.1212	1.423 ± 0.106	1.37 ± 0.06557

Values are mean ± S.D (n = 3 per group). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

Table 12: Quantitative data on absolute Organ weight of female rats in sub-acute toxicity study

Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovary
Control	1.77 ± 0.1044	0.55 ± 0.03606	1.153 ± 0.04933	1.473 ± 0.04163	4.333 ± 1.25	0.6367 ± 0.09609	1.38 ± 0.08888	0.36 ± 0.04583	0.11 ± 0.05196
PSP 200 mg/kg	1.667 ± 0.168	0.5667 ± 0.03215	1.173 ± 0.1563	1.123 ± 0.01155	4.737 ± 1.433	0.7133 ± 0.005774	1.463 ± 0.06658	0.3933 ± 0.08386	0.1167 ± 0.05508
PSP 400 mg/kg	1.84 ± 0.1513	0.46 ± 0.1136	1.13 ± 0.01	1.14 ± 0.01	3.293 ± 0.165	0.5833 ± 0.06658	1.393 ± 0.06658	0.43 ± 0.13	0.1433 ± 0.05508

Values are mean ± S.D (n = 3 per group). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.