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PRECLINICAL SAFETY EVALUATION OF TRADITIONAL SIDDHA FORMULATION PATTAI CHOORANAM IN WISTAR RATS

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ABSTRACT

Herbal 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. Although medicinal plants are widely used and assumed to be safe, however, they can potentially be toxic. Pattai chooranam (PTC) indicated in the siddha literature is one such herbal preparation which has multiple phytotherapeutics that can act synergistically in treating wide range of disorders. Hence the main objective of the present study is to evaluate the safety profile of PTC in rodent model through short and long term toxicity studies. 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 PTC were administered for 28 days and biochemical, hematological and histopathological parameters were evaluated.It was observed from the results of the present study that there were no significant changes was observed in acute and in subacute toxicity study rats with respect to behavior, gross pathology, body weight, and hematological and biochemical parameters. In conclusion from the data's of the present investigation that the LD50 value of PTC may be higher than 2000mg/kg and the drug was considered to be absolutely safe and has no hindrance with CNS, CVS and ANS of the experimental rats when treated at the dose of both the dose level of 200 and 400 mg/kg.

KEY WORDS: ***Siddha, Pattai chooranam, Safety, Acute, Sub-acute, Gross pathology, LD50, Hematological, Biochemical parameters***

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1. Introduction

Medicines derived from herbs have been used to treat human diseases since the dawn of medicine. Roughly 50% of new chemical entities introduced during the past two decades are from natural products. Recent technological advances have renewed interest in natural products in drug discovery [1]. Therefore, efforts should be directed towards isolation and characterization of the active principles and elucidation of the relationship between structure and activity. There are various medicinal plants and their derivatives (containing active chemical constituents, e.g., tannins and flavonoids) [2]. Furthermore, detailed analysis of the active constituents of natural drugs should be directed towards clinical relevance. Standardization is indispensable to maintain reproducible quality in biological evaluation. Although the clinical efficacy of these preparations is reported by traditional practices, they have not been scientifically validated.

Herbal medicine probably presents a greater risk of adverse effects and interactions than any other complementary therapy [3]. Serious adverse events after administration of herbal products have been reported, and in most cases, the herbs involved were self-prescribed and bought over the counter or were obtained from a source other than a registered practitioner [4].

Although therapies involving these agents have shown promising potential with the efficacy of a good number of herbal products clearly established, many of them remain untested and their use are either poorly monitored or not even monitored at all [5]. The consequence of this is an inadequate knowledge of their mode of action, potential adverse reactions, contraindications, and interactions with existing orthodox pharmaceuticals and functional foods to promote both safe and rational use of these agents. Since safety continues to be a major issue with the use of herbal remedies, it becomes imperative, therefore, that relevant regulatory authorities put in place appropriate measures to protect public health by ensuring that all herbal medicines are safe and of suitable quality [6].

Siddha system of medicine majorly relies on ancient traditional preparations for treating several infectious and non-communicable diseases. As per the vedic literature it has been provoked that this method of treatment has emerged from southern region of India and progressed though out the world [7]. There are versatile siddha formulations still left unexplored for its possible safety and efficacy. Pattai chooranam (PTC) indicated in the siddha literature is one such herbal preparation which has multiple phytotherapeutics that can act synergistically in treating wide range of disorders. Hence the main objective of the present study is to evaluate the safety profile of PTC in rodent model by acute and sub-acute repeated oral toxicity studies in accordance with standard regulatory guidelines.

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/167/2020

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 Pattai chooranam (PTC) 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 [8]. 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 PTC (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 assessment and interpretation [9].

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 [11]

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 [12]

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 PTC on Acute toxicity study

The dose of PTC 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 PTC in Acute toxicity study

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

3.3. Fecal Pellet consistency analysis of rats treated with PTC 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 analyzed 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 PTC on Sub-Acute toxicity study

The dose of PTC 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 PTC 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 PTC 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 PTC for 28 days in Sub-acute toxicity study

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

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

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

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

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

3.9.Effect of PTC 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 PTC at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 9.

3.10.Effect of PTC 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 PTC 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 PTC 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 PTC at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 12.

3.13. Effect of PTC 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.

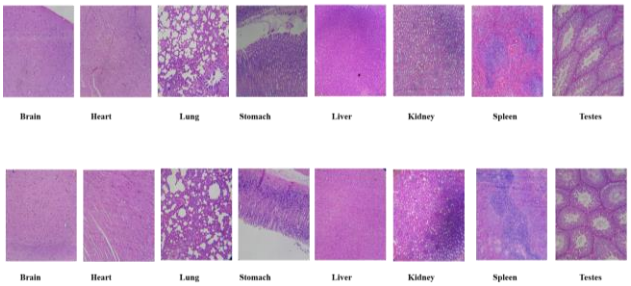

Figure 1: Histopathology of Male belongs to control and high dose treated group

3.14. Effect of PTC 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.

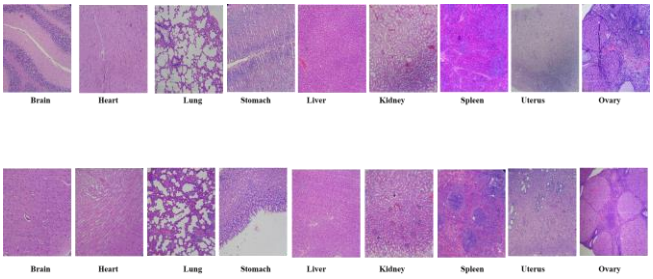

Figure 2: Histopathology of Female belongs to control and high dose treated group

4. Discussion

Health promotion, disease prevention and chronic disease management are proactive approaches to health care that stresses prevention at different points along the health care continuum. Health promotion and disease prevention strategies focus on keeping people well and preventing diseases from occurring. These strategies are referred to as primary prevention activities. Prevention is categorised into three levels [13].

Regulatory toxicity studies provides in-depth assessment to the researcher upon nature and origin of toxicity caused by the trial drugs. Guidelines implemented by OCED, ISO and WHO clearly emphasise the need and significance of preclinical toxicity studies in order to alleviate the possible ill effect of the drugs upon clinical application [14]. Rodents plays a vital role in arriving at the conclusion on wide range of toxicity signals upon administration over a period of time. Result of acute toxicity study signifies that the dose of PTC 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.

Providers of traditional medicines, such as physicians, nurses, and pharmacists, often have little training in and understanding of how herbal medicines affect the health of their patients. Many of them are also poorly informed about these products and how they are being used [15]. Adequate training is now very essential since most patients are almost often on other types of prescription or non-prescription medicines. In spite of the fact that the active involvement of orthodox healthcare professionals is continuously solicited and huge responsibility lies with them in terms of their valuable contributions to safety monitoring of medicinal products, it is also very important that all providers of herbal medicines are sufficiently empowered to play a role in monitoring safety of herbal medicines [16]. This, however, should be in collaboration with the orthodox healthcare professionals. For this to be effective, it would be essential to create an atmosphere of trust to facilitate adequate sharing of knowledge about the use and safety of herbal medicines. In fact, the education of healthcare professionals, providers of herbal medicines, and patients/consumers is vital for the prevention of potentially serious risks from misuse of herbal medicines.

Repeated oral toxicity study provides valued based information on long term adverse effect of the study drug with respect to the change in body weight, behavioural and other biochemical parameters including histological assessment. Investigation on the haematological parameters can be used to determine the extent of the deleterious effect of foreign compounds in herbal formulation on the blood constituents of an animal [17]. In the present study, the no significant difference in haematological parameters including RBC, WBC, Hb and other components of blood cell level following repeated daily dose treatment with siddha drug PTC could be an indication that it may not be toxic to the blood. This implies that the morphology and osmotic fragility of the RBC, as well as HB incorporation into the RBC, were not affected. This may also suggest that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following treatment with the drug intact [18].

Evaluation of serum biochemistry was done to identify the possible alterations in renal and hepatic functions affected by extract. Total protein, albumin, globulin, and total bilirubin also affecting the hepatocelluar and secretory functions of the liver. The lack of significant alterations in the levels of ALT, AST, ALP, creatinine, and uric acid, which are good indicators of liver and kidney functions [19]. In the present investigation it was observed that there is no significant chance in the serological profile of the control and drug treated rats with respect to BUN, Serum Creatinine, Total Bilirubin, SGOT, SGPT, total cholesterol, HDL, LDL, VLDL and TG. This denotes that administration of trial drug PTC did not alters the physiology and histomorphometery of any the internal vital organs.

5. Conclusion

In conclusion from the data's of the present investigation that the LD50 value of PTC may be higher than 2000mg/kg and the drug was considered to be absolutely safe and has no hindrance with CNS, CVS and ANS of the experimental rats when treated at the dose of both the dose level of 200 and 400 mg/kg. Oral administration of the drug Pattai chooranam can be considered safe as they did not exhibit any lethality nor adverse effects in the acute and sub-acute toxicity studies in both male and female rats subjected to investigation

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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 PTC- 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 Behaviour	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Colour	Yellowish
Urine pH	6-7
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)**
PTC 2000 mg/kg	183.5 ± 3.271	196.7 ± 3.011

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	**PTC**
Consistency	Soft
Shape	Round Headed
Color	Greenish
Mucous Shedding	Absent
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Control**	**PTC 200mg/kg**	**PTC 400mg/kg**
Consistency	Rigid	Soft	Soft
Shape	Oblong	Round Headed	Round Headed
Color	Greenish	Greenish	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	PTC 200 mg/kg	PTC 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	6 to 7	6 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	**186.3** ± 4.92	248.2 ± 11.2
PTC 200 mg/kg	187 ± 2.19	243 ± 12.2
PTC 400 mg/kg	188.2 ± 1.47	238.2 ± 7.8

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	14.2 ± 3.4	24.33 ± 1.96
PTC 200 mg/kg	13.83 ± 2.3	23.83 ± 3.06
PTC 400 mg/kg	14.6 ± 2.65	25.17 ± 2.78

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	5.967 ± 0.9309	6.283 ± 0.8841	743.8 ± 135.6	13.18 ± 1.258	15.75 ± 5.395	59.95 ± 4.028
PTC 200 mg/kg	7.367 ± 0.4457	7.633 ± 0.7685	548.8 ± 89.79	13.07 ± 2.113	17.28 ± 3.541	63.98 ± 3.568
PTC 400 mg/kg	5.867 ± 0.4131	7.517 ± 0.7885	736 ± 86.56	12.68 ± 1.065	16.33 ± 4.587	63.4 ± 4.576

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	2.667 ± 0.7891	1.3 ± 0.3286	0.3333 ± 0.5164	69.83 ± 8.709	2.3 ± 0.7071
PTC 200 mg/kg	3.05 ± 0.6091	1.7 ± 0.1414	0.1667 ± 0.4082	71.85 ± 7.93	2.55 ± 0.7609
PTC 400 mg/kg	2.533 ± 0.6532	1.433 ± 0.3386	0.1667 ± 0.4082	75.8 ± 9.016	4 ± 0.6099

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	16.17 ± 3.971	0.5667 ± 0.1366	0.3333 ± 0.1751	75.5 ± 35.85	46.33 ± 8.981
PTC 200 mg/kg	15.17 ± 4.119	0.7333 ± 0.1751	0.3833 ± 0.2229	99 ± 48.39	34.33 ± 7.257
PTC 400 mg/kg	15 ± 2.966	0.7667 ± 0.1211	0.3167 ± 0.09832	110.3 ± 20.55	38.67 ± 10.52

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	135.1 ± 22.7	70 ± 11.06	50.5 ± 24.95	14.62 ± 3.272	37.17 ± 8.519
PTC 200 mg/kg	127.7 ± 19.46	63.83 ± 16.15	37 ± 22.33	14.83 ± 3.387	36.33 ± 14.12
PTC 400 mg/kg	129.9 ± 29.78	59.5 ± 9.834	53.83 ± 27.76	16.55 ± 3.024	36.83 ± 11.69

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	1.75 ± 0.11	0.57 ± 0.08	1.22 ± 0.10	1.24 ± 0.10	4.34 ± 1.10	0.64 ± 0.12	1.22 ± 0.10	1.6 ± 0.04
PTC 200 mg/kg	1.763 ± 0.04163	0.5433 ± 0.02517	1.23 ± 0.08544	1.263 ± 0.1159	3.89 ± 0.7988	0.5433 ± 0.02082	1.47 ± 0.12	1.427 ± 0.1301
PTC 400 mg/kg	1.79 ± 0.0755	0.5033 ± 0.08145	1.2 ± 0.09539	1.51 ± 0.0866	4.313 ± 0.8053	0.5533 ± 0.03786	1.373 ± 0.1069	1.287 ± 0.1387

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	1.72 ± 0.1682	0.5333 ± 0.02082	1.143 ± 0.005773	1.193 ± 0.09452	5.177 ± 0.724	0.5167 ± 0.06658	1.183 ± 0.07095	0.3333 ± 0.04726	0.1 ± 0.04359
PTC 200 mg/kg	1.53 ± 0.11	0.5733 ± 0.07095	1.16 ± 0.04359	1.393 ± 0.05508	3.78 ± 0.09539	0.53 ± 0.01	1.463 ± 0.1106	0.4067 ± 0.1193	0.08333 ± 0.005774
PTC 400 mg/kg	1.69 ± 0.1587	0.53 ± 0.01	1.25 ± 0.1136	1.587 ± 0.1193	4.113 ± 0.6101	0.6033 ± 0.09292	1.39 ± 0.09	0.3767 ± 0.005773	0.07 ± 0.01

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.