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Preclinical Safety Evaluation of Siddha formulation *Guru Parpam* by Acute and Subacute repeated oral toxicity Studies in Rodents

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ABSTRACT

Since the beginning of human civilization, the Indian traditional system of medicine has been an essential component in the delivery of healthcare service and making it one of the oldest medical practices still in use today in the entire globe. India is the only country in the world that has its own system of traditional medicine that is recognized. These systems indicate a technique of establishing a healthy lifestyle while keeping to traditional and established views about the prevention of disease and promotion of health. They are founded on certain medical philosophies, and they represent a strategy that can be used to achieve these goals. Siddha system of medicine is flourishing across the world as it depicted with the physiology of rejuvenation and cure in altering the human physiology under diseased condition. The main aim of the present investigation is to validate the safety profile of the siddha formulation Guru Parpam (GP) by acute and sub-acute toxicity studies in accordance with regulatory guidelines. In the acute study, a single dose of 5000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses (250 and 500 mg/kg/day) of the test drug GP were administered for 28 days and biochemical, hematological and histopathological parameters were evaluated. Results of acute toxicity study revealed that the drug GP was found to be non-toxic at a dose of 5000 mg/kg b.w. In the sub-acute toxicity study there is no significant variations in body weight, hematological and serological parameters were observed in the experimental groups at the dose of 250 and 500 mg/kg with no mortality in both male and female rats subjected to the study. Vital organs such as the heart, brain, kidneys, liver, spleen, reproductive organs, etc. showed no changes in morphology or cytoarchitecture in histopathological studies. In conclusion, the acute and subacute toxicity study results show that the GP formulation is safe, and that the drug's long-term administration is unlikely to cause any adverse events.

KEY WORDS: ***Siddha, Guru Parpam, Acute, Sub-acute toxicity, Hematological, Serology, Histopathology***

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1. Introduction

Indian medicine takes a holistic approach to treatment, and the pharmacological modalities are based on natural materials derived from plants, animals, or minerals. This allows for the medicine to be more effective in treating a wide range of conditions [1]. As a result of this, there has been a recent uptick in interest in the practice of traditional medicine, which has historically been of assistance to the nation during pandemic crises caused by diseases such as the plague, cholera, and the Spanish flu, amongst others. As a consequence of this, it is essential in the current scenario to repurpose the traditional applications of traditional formulations in order to discover new treatment alternatives that may be used to tackle the currently devastating disease [2]. Since ancient times, numerous well-known and relatively risk-free traditional formulations of Indian medicine have been utilized for the purpose of treating a wide variety of diseases and conditions that affect humans.

Traditional medicines are believed to be safer than chemical products. Therefore, toxicity studies of such formulations do not usually receive as much attention as studies of chemical products. However, some traditional formulations are potentially toxic and may be harmful to human health [3]. Therefore, scientific knowledge towards oral toxicity of such formulation is much needed, which will not only help identify doses that could be used subsequently, but also to reveal the possible clinical signs elicited by agents under investigation.

The acute toxicity study may provide initial information on the mode of toxic action of an agent, acts as the basis for classification and helps in deciding the dose of drugs in animal studies. Moreover, if a high dose is found to be survivable, no further acute testing will be conducted [4]. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD50, therapeutic index and the degree of safety of a drug under investigation [5].

Siddha system of medicine is an ancient practice that substantially compensate the healthcare need of the people since several centuries. The principle of siddha treatment relies majorly on identifying the root causes of the disease emergence based on the state of vata, pitha and kaba. As the ideology of traditional medicine emphasize that change or alteration the tridosha paves way for metabolic changes in the body which would certainly call for many diseases [6]. The fundamental ingredients used for siddha preparations are from herbs, minerals, metals, animal products and from marine sources. Parpam being a fine powder are highly selective in action and hence predicting the safety index of this type of formulations are highly essential before subjecting the drug for human trials. The main aim of the present investigation is to validate the safety profile of the siddha formulation Guru Parpam (GP) by acute and sub-acute toxicity studies in accordance with regulatory guidelines. Followed by evaluation of serological, hematological and histopathological parameters in treated rodents.

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/XVII/182/2021

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 Guru Parpam (GP) at the dose of 5000mg/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 [7]. 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 to acclimatize for the 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 groups of 18 wistar albino rats (09 males and 09 females) 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 three group were treated with test drug GP (250 and 500 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 lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation [8].

2.4. Hematological analysis

Blood samples were analyzed using established procedures using 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 [9]

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 [10]

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 [11]

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 GP on Acute toxicity study

The dose of GP used for acute toxicity study is 5000mg/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 GP in Acute toxicity study

No significant change was observed in body weight of female rats treated with GP at the dose of 5000mg/ kg. The results were tabulated in Table 2.

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

Rats of control and GP 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 GP on Sub-Acute toxicity study

The dose of GP used for sub-acute toxicity study is 250 and 500 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 GP 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 GP at low and high dose of 250 and 500 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 GP for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with GP at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 6.

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

No statistically significant differences were recorded in hematological parameters of rats treated with GP at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 7.

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

No statistically significant differences were recorded in hematological parameters of rats treated with GP at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 8.

3.9. Effect of GP 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 GP at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 9.

3.10. Effect of GP 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 GP at low and high dose of 250 and 500 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 GP at low and high dose of 250 and 500 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 GP at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 11

3.13. Effect of GP 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. Histological examination of the vital organs examination of the organs belongs to control and treatment rats such as heart, liver, brain, spleen, kidneys, and lungs were found to be safe and does not exhibit any clinical signs of toxicity.

3.14. Effect of GP 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. Light microscopic observation of the sample belongs to control and drug treated rats reveals no sign of degeneration, inflammation, cytotoxicity and other signs of necrosis, which indicates the safety profile of the test drug.

4. Discussion

Drug of natural origin and their preparations have been revered for generations for their efficacy and lack of adverse effects. It's possible that this presumption played a significant role in the widespread application of these formulations among the rural populace. A lack of knowledge about the possible hazardous effects of long-term use and inadequate dosage monitoring by professionals are two of the main reasons why these formulations are frequently given for extended periods of time [12]. Therefore, there is a pressing need for research into oral toxicity, which will not only help determine future doses but also reveal potential clinical indicators provoked by drugs of interest.

Acute toxicity testing is carried out for the purpose of determining the effect of highest possible dose of test drug in experimental animal. It is advised, in most cases, that acute toxicity testing shall be carried out using female rats [13]. During the acute toxicological testing, the trial drug GP administered at the dose of 5000mg/kg, and the effects are monitored for a period of 14 days. Acute toxicity testing gives researchers the opportunity to calculate the 50% fatal dose (LD50) of an investigational substance. Historically, the LD50 was utilized as a measure of an indicative of acute toxicity. In order to determine the LD50, a huge number of animals are used, and the mortality rate during this process is very high. Because of these constraints, modified approaches were devised.

In sub-acute repeated dose toxicity study safety investigation of the test substance was evaluated by using repeated dosing strategy. Oral administration of the test drug takes place on a regular basis for a predetermined amount of time. The trial drug administered at regular intervals for the period of 28 days. In repeated dosage toxicity testing, the rodents utilized can be both male and female. There should not be a great deal of individual variance between the animals; the percentage of difference in weight that is acceptable is less than twenty percent. In this particular group, there is a "control" group as well as a "low and high-dose" group. It is important to keep track of baseline parameters, such as the behavioral and physiological characteristics of the animals. These will be useful in estimating the percentage changes that have occurred. In repeated dosage toxicity studies, it is absolutely necessary to evaluate the specifics pertaining to human safety. [14]

In sub-acute toxicity study treatment with GP at 250 and 500 mg/kg reveals no significant change in the body weight of the treated rats. The low and high dose selected for toxicity covers the average therapeutic dosage of the test drug GP in humans. Results of the study reveals that 28-day daily dose treatment with the GP elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation GP is safe at the tested doses over the observation period.

To assess the potential effects on hepatic and renal functions, serum haematology and clinical biochemistry studies were performed. Because of their vital role in maintaining life, the liver and kidneys are often tested as part of a medicine or plant extract's toxicity assessment [15]. Liver disorders and hepatotoxicity are associated with elevated levels of SGOT, SGPT, BUN and creatinine [16]. The fact that there were no statistically significant differences in liver enzymes and kidney biomarkers between male and female rats across all dosages is encouraging for the trial drug's safety.

It was observed from the results of the present investigation that, administration of GP at both the dose level in female and male rats for a period of 28 days produced no significant change in all blood parameters such as 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. Similarly there is no significant changes were observed in the serological investigation of the samples from the drug treated rodent with respect to the results of liver function, kidney function and lipid profile of experimental rats in both control and treatment group.

Relative organ weights is a sensitive indicator of the organ specific toxicity; researchers use this finding to define toxicity as significant alterations in the function of target organs [31]. The vital organs examination in the present investigation reveals that the organs such as heart, liver, brain, spleen, kidneys, and lungs were found to be safe and does not exhibit any clinical signs of toxicity. It was found that the test drug is relatively safe and there was no decrease in body and relative organ weights of the treated animals at both the dose level of 250 and 500mg/kg.

5. Conclusion

Siddha medicine attracts a great deal of attention due to its nontoxic nature and traditional use. Toxicity studies on siddha formulation commonly used to evaluate the possible health risk of the intrinsic chemical compounds in the preparation which could result in adverse effects. The results of the present study have strongly suggested that the siddha drug Guru Parpam is safe with wide safety margin established in both acute and sub-acute toxicity study further preclinical validation has to be carried out before clinical application of the drug for chronic ailment in humans.

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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 GP 5000 mg/ 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 to 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)**
GP 5000 mg/kg	183 ± 2.53	187 ± 7.69

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	**GP 5000mg/kg**
Consistency	Soft
Shape	Round Headed
Color	Pale greenish
Mucous Shedding	Absent
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Control**	**GP 250mg/kg**	**GP 500mg/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	Normal Saline	GP 250 mg/kg	GP 500 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	181.8 ± 1.4	222 ± 3.10
GP 250 mg/kg	186 ± 2.82	217.3 ± 2.80
GP 500 mg/kg	184.2 ± 3.18	212 ± 10.24

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	20.33 ± 3.26	23.67 ± 3.88
GP 250 mg/kg	17.3 ± 0.51	25.17 ± 3.76
GP 500 mg/kg	16.83 ± 2.13	25.5 ± 5.54

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 ($\times 10^6$ µl)	WBC ($\times 10^3$ µl)	PLT ($\times 10^3$ µl)	HGB (g/dl)	MCH (pg)	MCV (fl)
Control	5.3 ± 01.25	8.97 ± 1.78	725 ± 190.5	12.63 ± 2.20	21.27 ± 2.39	56.12 ± 3.22
GP 250 mg/kg	4.63 ± 0.417	7.48 ± 1.04	781.2 ± 98.38	11.52 ± 2.74	21.5 ± 1.69	61.07 ± 6.95
GP 500 mg/kg	5.21 ± 0.902	8.56 ± 2.47	707.5 ± 109.7	11.18 ± 3.13	20.82 ± 1.69	60.97 ± 5.36

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	2.22± 0.49	1.32±0.28	0.17±0.41	81.02± 6.63	3.60±0.73
GP 250 mg/kg	2.3 ± 0.456	1.3 ± 0.32	0.16 ± 0.1	81.1 ± 7.01	2.9 ± 4.46
GP 500 mg/kg	2.667 ± 0.436	1.433 ± 0.30	0.1667 ± 0.16	79.15 ± 9.702	3.78 ± 1.06

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	15.33 ± 3.27	0.82 ± 0.23	0.43 ± 0.08	100.30 ± 18.18	34.17 ± 7.68
GP 250 mg/kg	15.5 ± 4.46	0.75 ± 0.13	0.416 ± 0.14	95.83 ± 12.59	35.17 ± 7.36
GP 500 mg/kg	15.5 ± 3.45	0.76 ± 0.24	0.35 ± 0.104	91 ± 16.52	30.33 ± 6.37

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	126.10 ± 12.69	64 ± 8.74	47.50 ± 10.01	14.58 ± 2.35	38.17 ± 10.57
GP 250 mg/kg	120.8 ± 7.39	63.8 ± 7.25	42.83 ± 11.41	14.15 ± 2.93	39 ± 9.85
GP 500 mg/kg	122.3 ± 15.63	57.67 ± 10.15	49.5 ± 11.29	15.13 ± 3.00	36.5 ± 12.61

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	1.71 ± 0.07	0.62 ± 0.13	1.11 ± 0.23	1.34 ± 0.2	4.15 ± 0.09	0.39 ± 0.05	1.08 ± 0.03	1.71 ± 0.08
GP 250 mg/kg	1.76 ± 0.14	0.62 ± 0.04	1.46 ± 0.12	1.65 ± 0.11	5 ± 1.08	0.41 ± 0.09	1.25 ± 0.01	1.32 ± 0.35
GP 500 mg/kg	1.62 ± 0.11	0.63 ± 0.08	1.15 ± 0.18	1.55 ± 0.1	4 ± 0.43	0.34 ± 0.03	1.21 ± 0.09	1.88 ± 0.91

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 11: 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	1.407 ± 0.16	0.543 ± 0.10	2.193 ± 1.08	1.49 ± 0.06	4.62 ± 0.60	0.59 ± 0.12	0.98 ± 0.23	0.3 ± 0.08	0.076 ± 0.02
GP 250 mg/kg	1.493 ± 0.20	0.59 ± 0.09	2.56 ± 0.92	1.63 ± 0.32	4.553 ± 0.40	0.46 ± 0.03	1.07 ± 0.09	0.20 ± 0.12	0.08 ± 0.03
GP 500 mg/kg	1.657 ± 0.09	0.66± 0.03	1.68 ± 0.55	1.613 ± 0.33	6.017 ± 2.02	0.89 ± 0.53	1.12 ± 0.16	0.27 ± 0.13	0.04 ± 0.68

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.