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Acute and sub-acute (28-days) oral toxicity studies of Parangipattai Rasayanam

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Abstract

Purpose: Parangipattai Rasayanam is a poly herbal compound medicine mentioned in Pulippani vaithiyam-500.It is indicated for all kind of skin diseases, various ulcers, venereal diseases, peptic ulcer and arthritis[1]. **Aim and objective:** The objective of this study was to investigate the acute and sub-acute toxicity of PRM in Wistar rats. **Materials and Methods**: In the acute test, the limit test dose of 2000 mg/kg was administered to Wistar rats and then observed individually 1h post-dosing, and at least once daily for 14 days. Sub-acute toxicity was evaluated after administering daily oral doses of 300, 600 and 900 mg/kg body wt., for 28 days to the rats, Biochemical and hematological assessments as well as body and relative organ weights of the rats were carried out. **Results:** The limit dose of 2000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. In the sub-acute tests, the results did not show any treatment–related abnormalities in terms of hematological and biochemical parameters. The weekly body and organ weight of the rats showed no significant differences between the control and the rats treated with the PRM. **Conclusion:** Our results suggest that the PRM is relatively safe when administered orally in rats.

Keywords: Parangipattai Rasayanam, Acute and sub-acute toxicity, Biochemical parameters, Haematological analysis, Wistar rats.

1. Introduction

Siddha system of medicine is one such ancient traditional system of India and practiced mostly in its southern part for treating various diseases including even chronic conditions[2.] Siddha system is an impressive and ancient Indian medical system, which, historically, was not popularized due to the secrecy maintained by the *Siddharkal*. Medicine as everyone knows is not merely a science but an art as well. The traditional system of medicine became significantly more popular all over the globe because of the less toxic and has no side effects. The mode of preparation and plant used in traditional medicine varies from place to place and in addition acceptance of traditional medicines in development world is sharply increasing. In Siddha system of medicine, the drug sources are mainly obtained from plants, animal products, minerals and metals. In this present study, an attempt was made systematically to study the toxicity profile of the Siddha drug.

The types of toxicity tests which are routinely performed by pharmaceutical manufactures in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 the dose which has proved to be lethal (causing death) to 50% of the 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 all compounds. (Akhila *et al*., 2007). Acute toxicity is produced after administration of a single dose or multiple doses in a period not exceeding 24 hours, up to a limit of 2000 mg/k g. Objective of acute toxicity studies is to identify a dose causing major adverse effects and an estimation of the minimum dose causing lethality (Robinson *et al*., 2007). In recent times there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines (Steve *et al*., 2009). The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs.

2. Materials and Methods

2.1. Procurement and Authentication of Raw Drugs:

The required raw drugs for the preparation of PRM were purchased from a reputed country shop. The raw drugs were identified and authenticated by medicinal botanist of National Institute of Siddha. All the raw drugs were made into a fine powder. This powder is then mixed with sugar, honey and ghee to the consistency of Rasayanam.

2.2. Preparation of Parangipattai Rasayanam:

The raw drugs were purified as mentioned in Siddha literature[3,4]. Parangipattai Rasayanam was prepared using the procedure described in Siddha literature[1].

2.3. Animal Care and Husbandry:

The study protocol involving animals was reviewed and approved by Institutional Animal Ethical Committee (IAEC), KMCH College of pharmacy, Kovai estate, with the experimental protocol number IAEC NO: KMCRET/MD(S)/9/2014-15. Experiments were performed as per the instructions prescribed by the Committee for the Purpose of conduct and Supervisions of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Male and female Wistar albino rats, (140–160 g) obtained from Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore, were housed in the animal house KMCH College of pharmacy, Kovai estate. Each group of rats was separately housed in polypropylene cages in a well ventilated room under an ambient temperature of 22±3°C and 30-70% relative humidity, with a 12-h light/dark artificial light cycle. . They were provided with food (SaiDurga Animal Feed, Bangalore) and purified water ad libitum. All the animals were acclimatized to the laboratory conditions at least for 7 days prior to experimentation.

Acute toxicity studies

The acute oral toxicity study was performed in accordance with Organization for Economic Cooperation and Development (OECD) test guideline 423[5]. The limit test dose of 2000 mg/kg was used as stipulated in Organization for Economic Cooperation Development (OECD) guidelines. The test drug suspension was administered at a dose of 2000 mg/kg once orally to the fasted rats. In the experiments the observations like body weight, clinical signs and gross pathology were common and are as follows

a. Mortality or morbidity was noted.
b. Following test item administration weekly body weight was recorded.
c. Clinical signs such as Lethality, Convulsion, Tremor, Straub tail, Sedation, Excitation,Abnormal gait (rolling), Abnormal gait (tiptoe), Jumps, Motor coordination, Loss of balance, Fore paw treading, Writhes, Piloerection, Stereotypies (chewing), Stereotypies (Head movements), Head twitches, Scratching, Respiration, Aggressiveness, Fear, Reactivity to touch, Muscle tone, Loss of righting Reflex, Ptosis, Exophthalmos, Loss of grasping, Akinesia, Catalepsy, Loss of traction, Loss of corneal reflex, Analgesia, Defecation, Salivation,

Lacrimation, Others: were observed at approximately 30 mins, 1hr, 2hr and 4hr on day 1 and daily thereafter for 14 days.

At the end of 14 days, the experimental animals were necropsied and investigated for gross pathological examination.

Sub-acute toxicity test:

Justification for Dose Selection:

As stated in results of acute toxicity studies in wistar rats indicated that PRM was nontoxic up to the maximum dose level of 2000 mg/kg body weight. On the basis of these results, the doses selected for the study was 300 mg/kg, 600 mg/kg and 900 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose

Repeated-dose oral toxicity study was carried out according to OECD guideline 407[6]. The animals were divided into six groups of 10 animals each (5 males and 5 females). Group 1 received distilled water and served as control. Groups II, III and IV received test drug suspensions, (PRM with distilled water)doses of 300,600 and 900 mg/kg body wt, respectively. Group V (control) and Group VI test drug suspensions high dose (900 mg/kg) were included in the satellite study to determine the delayed occurrence, or persistence of, or recovery from toxic effects. The PRM administered daily for 28 days the same time daily and observed at least twice daily for morbidity and mortality. Bodyweights of the animals were evaluated weekly.

The satellite groups were scheduled for follow-up observations for the next 14 days without PRM administration. The test drug suspensions (PRM with distilled water) were freshly prepared every day and administered orally (gavage) once daily for 28 consecutive days. Initial body weight of all the groups was recorded. The animals were monitored closely for signs of toxicity throughout the course of study. Appearance and behavior pattern were assessed daily and any abnormalities in food and water intake were registered.

On the 29th day, after an overnight fast, the rats were anaesthetized with ketamine and blood sample for heamatological and biochemical analysis were collected into tubes with and without EDTA, respectively. Haemoglobin, Red blood cell count, White blood cell count, Mean Corpuscular Haemoglobin (MCH), Packed Cell Volume (PCV) was determined using fully automated haematology analyzer. Biochemical analysis was performed on serum obtained after centrifugation of total blood (without anticoagulant) at 2500 rpm for 15 min. Biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, alkaline phosphatase, glucose, total proteins and urea also determined.

Histopathology

Necropsy was done in all animals on 29th dayexcept the satellite groups for which it was done on 42nd day. After blood collection, all the animals were euthanized for gross pathological examinations of all major internal organs. The organs such as brain, heart, liver, kidneys, were weighed and relative organ weights were calculated. The organs were fixed in 10% neutral buffered formalin, trimmed and a 5µ thickness of tissue sections were stained with hematoxylin and eosin for histopathological investigation.

Statistical analysis

All the values are expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA followed by Dunnet-t test. P values < 0.05 were considered significant.

Results and Discussion

Acute toxicity studies

The limit dose of 2 g/kg did not cause mortality or any sign of acute toxicity in the three rats dosed for a short period (48 h) and long period (14 days).No behavioural changes and death were observed at the end of the treatment. Similarly, no significant differences in bodyweight were observed between control and treated groups during this period (see Table1).

Table 1: Dose finding experiment and its behavioral Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	2000mg/kg bw	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+

1. Alertness 2. Aggressiveness 3.Piloerection 4.Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9.Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18.Writhing 19.Respiration 20. Mortality.

Sub acute toxicity study

There were no treatment-related toxicity signs and mortality observed in both sexes of rats treated at 300, 600 and 900 mg/kg orally for a period of 28 days and in the satellite group of rats. No significant difference in body weight gain was observed between control and treated groups during the study (Table 2). Feed and water consumption of PRM treated groups were found to be In significant in both the sexes when compared to control. Hematological parameters such as heamoglobin, red blood cells, white blood cells, mean corpuscular heamoglobin, were found to be well within the clinical range of rats in experimental groups (Table3). There were no significant difference in plasma biochemical profile such as glucose, total cholesterol, triglycerides, total protein, alkaline phosphatase, creatinine, blood urea (Table 4) observed between control and treated groups. The levels of liver marker enzymes like SGOT and SGPT were found to be well within the clinical range of rats in PRM treated groups (Table4). There were no significant differences in organ weight of brain, heart, liver, lungs, kidneys recorded between the control and PRM groups (Table5). In our study, histopathological examinations in control and high dose group revealed no abnormalities. There were no hematological, biochemical and histopathological alterations observed with PRM administration even at 900 mg/kg/day in rats for a period of 28 days compared to control. The No-Observed Adverse Effect Level (NOAEL) of PRM was estimated to be greater than 900 mg/kg/day in rats. Hence, it can be concluded that PRM is safe for oral administration.

Table 2: Effect of Paranipattai Rasayanam on Body weight of experimental Wistar rats in repeated oral toxicity study

Treatment	1st day	7th day	14th day	21st day	28th day	35th day	42nd
Control	144.90±0.90	149.00±1.94	153.70±1.92	158.40±1.82	162.10±1.73	-	-
300mg /kg p.o/day	143.50±0.82	147.70±0.84	151.80±0.80	155.90±0.90	160.20±0.81	-	-
600mg /kg p.o/day	143.80±0.65	146.80±0.90	151.40±0.73	155.90±0.99	159.90±1.22	-	-
900mg /kg p.o/day	143.60±0.62	146.70±0.75	150.40±0.81	155.60±0.86	159.70±0.80	-	-
Satellite group							
Control	144.20±0.90	148.20±0.53	153.20±0.66	157.80±0.55	161.90±0.69	167.00±0.77	171.40±0.97
900mg /kg p.o/day	144.70±0.73	153.40±0.60	158.20±0.73	158.40±1.82	162.00±0.82	167.20±0.94	171.00±1.05

Comparison was made between vehicle control and test groups using the one-way ANOVA test followed by Dunnetts test. Values are mean ± SEM for 10 rats in each group ($P < 0.05$).

Table 3: Effect of Paranipattai Rasayanam on Hematological Parameters of experimental Wistar rats in repeated oral toxicity study:

Treatment	Hematological parameters						
	WBC (10^3/uL	RBC (10^6/uL)	Hgb g/DL	PCV %	MCH pg	Lymphocyte %	Monocyte %
Control	9.81±0.36	6.86±0.10	14.50±0.63	47.03±1.78	19.84±0.56	83.30±1.22	2.60±0.27
300mg/kgp.o/ day	9.75±0.28	7.03±0.15	14.98±0.70	49.40±1.42	19.51±0.60	81.60±1.07	2.50±0.27
600mg/kg p.o/day	9.95±0.40	7.27±0.21	14.87±0.24	45.68±1.92	19.65±0.42	87.90±0.84	2.40±0.31
900mg/kgp.o/ day	10.04±0.37	8.15±0.28	14.73±0.52	44.87±1.02	19.55±0.49	84.30±0.99	2.30±0.26
Satellite group							
Control	9.90±0.39	6.90±0.09	14.32±0.25	44.13±1.38	18.91±0.28	82.10±0.84	2.30±0.33
900mg /kg p.o/day	9.66±0.26	7.58±0.30	15.42±0.23	47.85±1.05	20.16±0.66	83.70±1.24	2.50±0.27

Comparison was made between control and test groups using the one-way ANOVA test followed by Dunnetts test. Values are mean ± SEM for 10 rats in each group *(P* < 0.05).

RBC: red blood corpuscles; HGB: hemoglobin; PCV: packed cell volume; MCH: mean corpuscular hemoglobin; WBC: white blood cells.

Table 4: Effect of Paranipattai Rasayanam on biochemical parameters of experimental Wistar rats in repeated oral toxicity study:

Treatment	Biochemical parameters								
	Glucose mg/dl	TGL Mg/dl	Cholesterol mg/dl	SGPT U/I	ALP U/I	SGOT U/I	T. Protein g/dl	Creatinine mg/dl	Urea Mg/dl
Control	90.60±1.82	73.75±2.68	97.95±3.26	62.02±3.15	287.03 ±10.04	147.77± 2.79	6.96±0.34	0.91± 0.03	32.85 ±2.69
300mg/kg p.o/day	92.00±1.87	75.67±1.77	100.33±3.59	68.97±2.61	282.85 ±14.91	154.89± 3.03	7.35±0.31	0.68± 0.06	31.51 ±3.27
600mg/kg p.o/day	87.50±2.11	76.19±2.35	98.27±3.52	65.19±3.60	285.86 ±8.95	150.17± 3.60	7.49±0.45	0.86± 0.04	37.70 ±0.72
900mg/kg p.o/day	89.10±2.70	72.88±1.92	96.49±2.53	62.75±1.94	290.03 ±10.43	152.33± 3.60	7.24±0.30	0.67± 0.05	30.06 ±1.30
Satellite group									
Control	93.00±1.81	74.21±2.75	101.40±2.40	64.47±3.78	286.12 ±7.29	151.15± 4.68	7.97±0.36	0.90± 0.04	30.61 ±1.75
900mg/kg p.o/day	92.20±2.67	72.70±1.48	98.37±2.61	64.05±2.94	287.51 ±8.90	160.96± 5.54	7.29±0.42	0.62± 0.05	28.38 ±1.00

Comparison was made between vehicle control and test groups using the one-way ANOVA test followed by Dunnetts test. Values are mean ± SD for 10 rats in each group *(P*<0.05).

TGL: triglycerides, SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase, ALP:alkaline phosphatase.

Table 5: Effect of Paranipattai Rasayanam on Organ weight of experimental Wistar rats in repeated oral toxicity study

Treatment	Relative organ weight (g %)						
	Brain	Lungs	Heart	Liver	Kidney	Sex organs	
						Testis	Ovaries
Control	1.18±0.03	0.87±0.03	0.43±0.01	3.82±0.09	0.96±0.02	1.08±0.04	0.05±0.00
300mg PRM/kg p.o/day	1.22±0.02	0.88±0.03	0.43±0.01	3.77±0.09	0.97±0.02	1.07±0.06	0.05±0.00
600mg PRM/kg p.o/day	1.24±0.02	0.85±0.02	0.42±0.01	3.92±0.12	0.98±0.02	1.08±0.05	0.05±0.00
900mg PRM/kg p.o/day	1.23±0.02	0.88±0.02	0.43±0.01	4.00±0.14	0.97±0.03	1.08±0.03	0.05±0.01
Satellite group							
Control	1.19±0.01	0.89±0.03	0.41±0.01	3.62±0.15	0.96±0.02	1.06±0.05	0.06±0.01
900mg PRM/kg p.o/day	1.18±0.02	0.88±0.05	0.43±0.01	3.87±0.15	0.96±0.02	1.08±0.03	0.05±0.01

Comparison was made between control and test groups using the one-way ANOVA test followed by Dunnett's test. Values are mean ± SEM for 10 rats in each group (P <0.05).

Discussion

Since PRM is in clinical use for Psoriasis treatment for more than 20 years, a limit test was performed in acute oral toxicity study. According to the OECD test guideline 423 when there is information in support of low or non-toxicity and immortality nature of the test material, then the limit test at the highest starting dose level (2000 mg/kg body weight) was conducted. There were no mortality and toxicity signs observed at 2000mg/kg. PRM can be classified under category-5 and LD_{50} value was greater than 2000mg/kg in accordance with Globally Harmonized System of Classification and Labeling of chemicals and this provides us a direct relevance for protecting human and animal health. Therefore, it can be concluded that Parangipattai Rasayanam when administered at single dose is non-toxic and can be used safely in oral formulations.

A 28-day repeated oral toxicity study was performed followed OECD test guideline 407in both male and female wistar albino rats. Since examination of clinical signs plays major role in toxicological testing[7], mortality and morbidity were recorded twice a day throughout the study. PRM did not produce any alterations in feed and water consumption and this reveals that it did not adversely affect the basic metabolic processes of the experimental animals. The haemopoietic system serves as important target for toxic chemicals and is a sensitive index for pathological conditions both in humans and animals[8]. In the present study, treatment with PRM did not produce any alteration in hematological parameters (i.e. RBC, WBC, haemoglobin, etc.), which indicate that PRM did not affect blood cells nor their production. Clinical biochemistry and hematological data holds significant role in determining the toxicity induced by drugs. Transaminases (SGOT and SGPT) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs. Any elevation pertaining to these enzymes indicate their outflow into the blood stream due to damage in liver parenchymal cells.

There were no changes in the SGPT and SGOT levels which reveal that PRM did not affect liver function/or metabolism. In the present study, there were no treatment related abnormalities in renal function and other biochemical parameters

suggesting that PRM is non-toxic. Histopathological studies provide supportive evidence for biochemical and haematological observations. The organ weights were found to be non-significant between the control and PRM treated rats. No abnormality was recorded with respect to gross or histopathological examinations of all organs examined. Since there were no signs of toxicity with respect to hematology, clinical chemistry, organ weight, gross and histopathological examinations noted in PRM satellite group, it can be inferred that PRM will not produce delayed onset of toxicity. Based on these results, the No Observed Adverse Effect Level (NOAEL) of "PRM" is greater than 900 mg/kg/day.

Conclusion

In present days, world's focus turns to the herbal medicine because of the side effect of other drugs. Siddha medical system is very powerful among the Indian system of medicines.In accordance with Globally Harmonized System of Classification and Labeling of chemicals, PRM can be classified as Category 5. Based on 28 day repeated dose toxicity study, NOAEL of PRM is greater than 900 mg/kg/day. The present investigation substantiates, at least in part, the safety of PRM, which was found to be in line with the long history of its use in Siddha system of medicine.

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