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Research Article

ACUTE AND SUB ACUTE TOXICITY STUDY OF GANDHAGA BAGA PARPAM IN RATS

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ABSTRACT

Gandhaga baga parpam is effective to treat skin diseases in Siddha system of medicine. Safety of Gandhaga baga parpam was studied as per OECD guideline 423 and 407 in wistar albino rats. Acute toxicity study revealed LD_{50} of Gandhagabaga parpam was 1000 mg/kg b. wt. In 28 days repeated oral toxicity study, no mortality and morbidity were observed, biochemical and hematological analysis results showed decreases in SGOT, SGPT, ALP, Hb and Na in 100 mg/kg. b. wt and 200 mg/kg. b. wt treated group compared with control group. Some changes were observed in renal parameters and lipid profile. All the vital organs are normal histology except liver, stomach in treatment group. 100 mg/kg of test drug treated group liver showed chronic venous congestion and 200 mg/kg test drug treated group liver showed chronic venous congestion, peri-portal lymphocytic infiltration and stomach showed hyperplasic of gastric mucous with focal surface ulceration. Based on these NOAEL and LOAEL of Gandhaga baga parpam was 100 mg/kg. bwt and 200 mg/kg.bwt.

Keywords: Gandhaga baga parpam, Siddha medicine, Acute and Sub acute toxicity study.

INTRODUCTION

Siddha system is an impressive and ancient Indian Medical system. The Siddha system of medicine mentioned for various ailments are the essence of Siddhars divine knowledge and intuitions. Siddha medicine should be subjected to standardization, pharmacological and toxicological analysis for global acceptance. Sulphur places an important role in Siddha system of Medicine to treat thol noigal (Skin diseases)[1]. Gandhaga baga parpam formulation contains sulphur and sodium chloride. Gandhaga baga parpam is mentioned to treat skin diseases and venereal diseases[2]. While research in plants has received considerable attention, the mineral preparations have relatively neglected. Studies on the role of elements in health and disease have now become of global importance with rise of research activity in the two decades[3,4]. So Gandhaga baga parpam was undertaken to access its safety through toxicological studies as per OECD guideline in rats[5,6].

Aim and objectives

To find the toxicity profile of Gandhaga baga parpam through Acute and sub-acute toxicity study

MATERIAL AND METHODS

Toxicity Studies

Gandhaga baga parpam was prepared as per Siddha literature[2] and subjected to toxicity study as per OECD guideline 423 and 407 with slight modification after IAEC approval.

Experimental Animals

Adult wistar albino rats of either sex weighing 80-120 g were used (VELS College of Pharmacy, Chennai). The animals were fed with standard rat diet (Sai meera foods, Bangalore) had free access to water under well ventilated condition of 12 hours light cycle. They were kept in a standard poly propylene rat cages with steel top grill as bedding and were adapted to laboratory conditions for 7 days prior to the experiments.

Solvent used for Toxicity Study

2 % Carboxyl methyl cellulose used as a solvent for toxicity study.

Acute Toxicity Study

3 Male and 3 Female wistar rats were dosed on a stepwise procedure using the fixed doses of 50, 100, 250, 500, 1000 and 2000 of test drug test drug by orally using gastric gavage. The control group received an equal volume of the water. The principles of laboratory animal care were followed. The animals were fasted prior to dosing according OECD guidelines, food but not water was withheld for 16 – 18 hours. Following period of fasting, the animals were weighed to determine the appropriate weight (mg/kg) of the test drug for making various concentrations for each of the experiments. Observations were made and recorded systematically 1, 2, 4, and 24 hours after administration. The visual observations included Skin changes, Mobility, Aggressively, Sensitivity to sound and pain, as well as Respiratory movements, etc. Finally, the number of survival animals was noted after 24 h and these animals were then maintained for a further 13 days and observations made daily to record signs of toxicity and death if any. The LD_{50} values and toxicity range of Gandhaga baga parpam was determined. The toxicological effect was assessed on the basis of mortality.

28 Days Repeated Oral Toxicity Study

The repeated dose 28 days oral toxicity was carried out in wistar albino rays according to OECD guideline – 407. A total of eighteen apparently healthy rats of either sex was used. Eighteen rats were randomly distributed into three groups with 3 Males and 3 Females/group. Randomization was done using a random number table as per the standard procedure. Group I received water and served as control. Group II and III received test doses of 100 and 200 mg/kg respectively of test drug suspended in 2% CMC by orally. The animals were dosed daily for a period of 28 days and the doses were given at similar time each day. Adjustment was made as necessary to maintain a constant dose level in term of animal body weight. Animals were observed at least twice daily for mortality and morbidity. Weekly body weight change of each rat was recorded throughout the experiment period. Weekly food and water consumption of rats were recorded for the entire duration of the experiment. After 28 days all surviving animals were fasted overnight. The animals were anaesthetized on the 29th day with ether and blood sample for hematological and biochemical analysis was collected from the orbital sinus into coated EDTA tube and non-heparinized centrifuge tubes respectively. After blood collection all the animals were sacrificed and the brain, heart, lung, liver, spleen, pancreas, stomach, small intestine, kidney, testis, ovary, femur and skin were collected and preserved on 10% formalin saline solution and histopathological study was done.

Statistical Analysis

The results are presented as mean ± S.D. and the statistical analyzed by means of an analysis of variance followed by Dunnett's multiple comparison test. P values less than 0.05 were considered as significant.

RESULTS

Table 1: Increased dose level finding experiment and its science of toxicity- Acute Toxicity Study

Treatment group	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I	50	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	N	-
II	100	+	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	N	-
III	250	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	+	+	N	-
VI	500	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	+	+	N	-
V	1000	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	+	+	N	+++
VI	2000	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	+	+	N	++++

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Increased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle Relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exopthalmus 17. Diarrhoea 18. Writhing 19. Respiration 20. Number of Deaths (Mortality). N –Normal respiration

Table 2: Mean body weight of albino rats after 28 days treatment with Gandhaga Baga Parpam

Dose (mg/kg/day)	Mean body weight (gram/week)			
	I week	II week	III week	IV week
100 mg/kg	96.6 ± 6 3.073	93.33 ± 2.108	75.83 ± 2.00**	65 ± 2.58**
200 mg/kg	112.5 ± 8.73	107.5 ± 8.827	82.5 ± 4.95*	77.5 ± 3.35**

Values are mean of 6 animals ± S.D. (ANOVA followed by Dunnett's test).*p < 0.05; **p < 0.01 vs I week

Table 3: Mean food intake of albino rat after 28 days treatment with Gandhaga baga parpam

Dose (mg/kg/day)	Mean food intake (gram/week)			
	I week	II week	III week	IV week
100 mg/kg	63 ± 1.789	48.8 ± 2.245**	35.4 ± 1.913**	23.6 ± 1.568**
200 mg/kg	63.6 ± 1.568	48 ± 4.637**	25 ± 1.581**	22 ± 1.22588**

Values are mean of 6 animals ± S.D. (ANOVA followed by Dunnett's test).*p < 0.05; **p < 0.01 vs I week

Table 4: Mean water intake of albino rat after 28 days treatment with Gandhaga baga parpam

Dose (mg/kg/day)	Mean water intake (ml / week)			
	I week	II week	III week	IV week
100 mg/kg	42.4 ± 1.122	40.1 ± 2.96	25 ± 2.739**	22 ± 1.225**
200 mg/kg	39 ± 2.915	46.2 ± 2.835	22 ± 2.55**	17.6 ± 1.122**

Values are mean of 6 animals ± S.D. (ANOVA followed by Dunnett's test) *p<0.05;**p<0.01 vs I week.

Table 5: Hematological parameters after 28 days treatment with Gandhaga baga parpam

Parameters	Control	100 mg/kg	200 mg/kg
Total RBC (millions/mm^3)	5.68 ± 0.60	5.8 ± 0.10	2.1 ± 0.12
Hb (gm)	20.16 ± 0.703	12.16 ± 0.477**	9.68 ± 0.322**
Leukocyte (× 10/mL)	6276.66 ± 45.94	4400 ± 44.72**	4075 ± 38.18**
PCV (%)	54.16 ± 1.014	40 ± 1.390**	30.8 ± 1.014**
MCV (cubic micro)	88.66 ±1.382	81.66 ± 0.56**	145.83 ± 1.493**
MCH (pg)	30.16 ± 0.60	19.83 ± 0.60**	48.83 ± 1.44**
MCHC (%)	33.5 ± 0.763	30 ± 0.730	34.83 ± 0.542
Platelets (thousands/micro liter)	4.066 ± 0.11	3.4 ± 0.085	3.16 ± 0.105
Differential Leukocyte Count (%)			
Neutrophil	35.83 ± 0.703	43.83 ± 1.249	26.16 ± 1.13
Basophil	-	-	-
Eosinophil	3.21 ± 0.22	3.18 ± 0.19I	3 ± 0.22
Lymphocyte	60.5 ± 0.846	52.33 ± 0.760	70.33 ± 1.054
Monocyte	2.33 ± 0.24	2.11 ± 0.15	1.3 ± 0.16
ESR			
½ - hr	2.016 ± 0.065	4.05 ± 0.076	2 ± 0.093
1- hr	4.18 ± 0.079	6.06 ± 0.122	4.05 ± 0.108

Values are mean of 6 animals ± S.D. (ANOVA followed by Dunnett's test).*p < 0.05; **p < 0.01 vs control

Biochemical parameters of albino rats after 28 days treatment with Gandhaga baga parpam

Table 6: Liver function test and Blood glucose

Parameters	Control	100 mg/kg	200 mg/kg
Total Bilirubin (mg/dL)	0.24 ± 0.014	0.58 ± 0.023	0.67 ± 0.025
Direct Bilirubin (mg/dL)	0.7 ± 0.057	0.42 ± 0.006	0.30 ± 1 0.008
Indirect Bilirubin (mg/dL)	0.13 ± 0.004	0.19 ± 0.007	0.4 ± 0.013
ALP (U/L)	56.5 ± 0.76	67 ± 1.75**	29 ± 0.93**
SGOT (U/L)	178.16 ± 2.75	200 ± 4.139**	207 ± 3.987**
SGPT (U/L)	58.16 ± 1.195	31.83 ± 0.980 **	45 ± 0.730
Total protein (g/dl)	8.44 ± 0.235	8.25 ± 0.51	7.73 ± 0.27
Albumin (g/dl)	2.61 ± 0.087	2.85 ± 0.09	2.45 ± 0.08
Globulin (g/dl)	5.98 ± 0.25	7.69 ± 0.26	4.96 ± 0.19
A/G Ratio (g/dl)	0.441 ± 0.011	0.33 ± 0.013	0.473 ± 0.012
GGT (U/L)	12 ± 0.57	18.33 ± 1.05	23.5 ± 0.76
Blood glucose (mg/dl)	122.5 ± 5.36	130.33 ± 0.881	133.5 ± 2.865

Values are mean of 6 animal's ± S.D. (ANOVA followed by Dunnett's test). *p < 0.05; **p < 0.01 vs control

Table 7: Renal Function Test

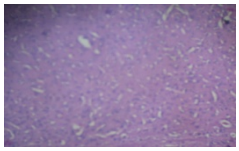
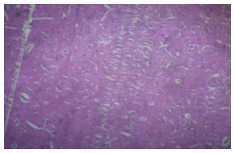
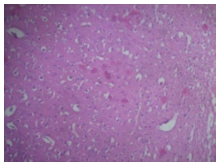
Parameters	Control	100 mg/kg	200 mg/kg
Urea (mg/dl)	31.5 ± 0.84	71.5 ± 1.3888	56 ± 0.68**
Creatinine (mg/dl)	1.08 ± 0.041	0.58 ± 0.047	0.5 ± 0.036
Uricacid (mg/dl)	17.75 ± 0.42	3.26 ± 0.066**	7.06 ± 0.21**
Na (m.mol)	142.5 ±1.56	135.5 ± 1.58**	155.5 ± 2.553**
K (m.mol)	1.99 ± 0.052	3.25 ± 0.067	5.65 ± 0.10
Cl (m.mol)	208 ±1.065	91.5 ± 0.76**	94.66 ± 0.760**

Values are mean of 6 animal's ± S.D. (ANOVA followed by Dunnett's test) *p < 0.05; **p < 0.01 vs control

Table 8: Lipid profile

Parameters	Control	100 mg/kg	200 mg/kg
Total cholesterol (mg/dL)	95 ± 2.366	74.16 ± 1.579**	64.5 ± 0.991**
HDL (mg/dL)	22.5 ± 0.991	29.33 ± 0.88*	28 ± 0.856*
LDL (mg/dL)	50.66 ± 1.358	20.83 ± 0.703**	19.66 ± 0.421**
VLDL (mg/dL)	23 ± 1.065	24.5 ± 1.76	25.16 ± 0.654
Triglycerides (mg/dL)	119.16 ± 3.683	122.66 ± 1.256	125.16 ± 2.023
TC/HDL ratio (g/dL)	4.486 ± 0.114	2.483 ± 0.098	2.505 ± 0.116*

Values are mean of 6 animal's ± S.D. (ANOVA followed by Dunnett's test). *p < 0.05; **p < 0.01 vs control

Group I- water	Group II-100 mg/kg	Group III- 200 mg/kg
Brain	Brain	Brain
		
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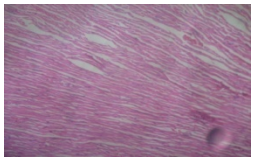	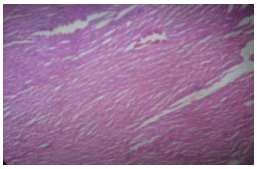	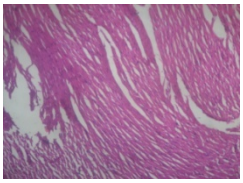
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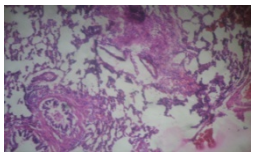	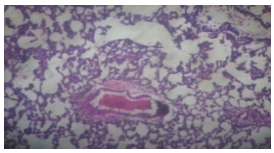	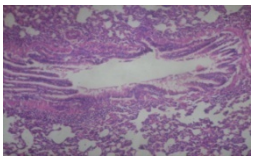
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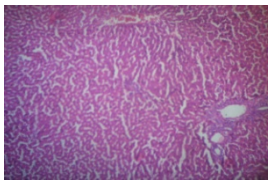	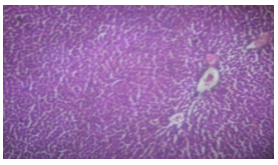	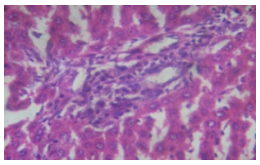
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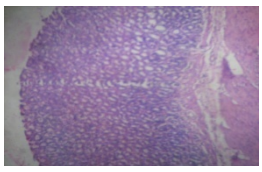	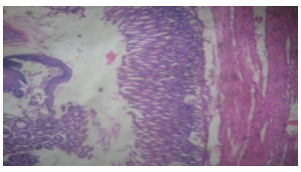	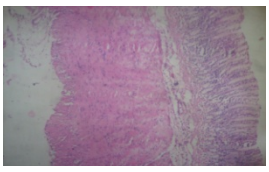
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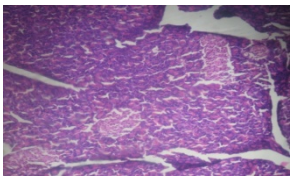	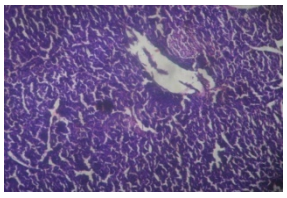	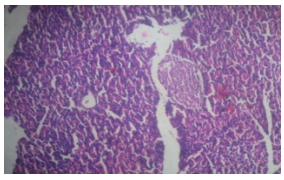
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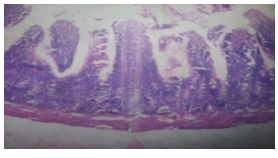	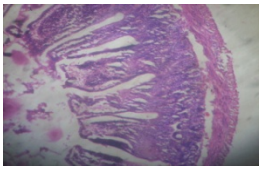	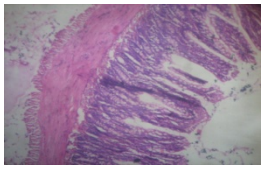
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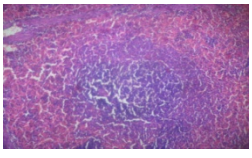	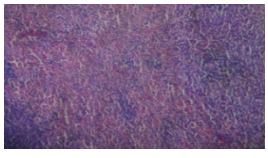	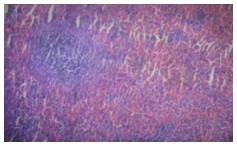

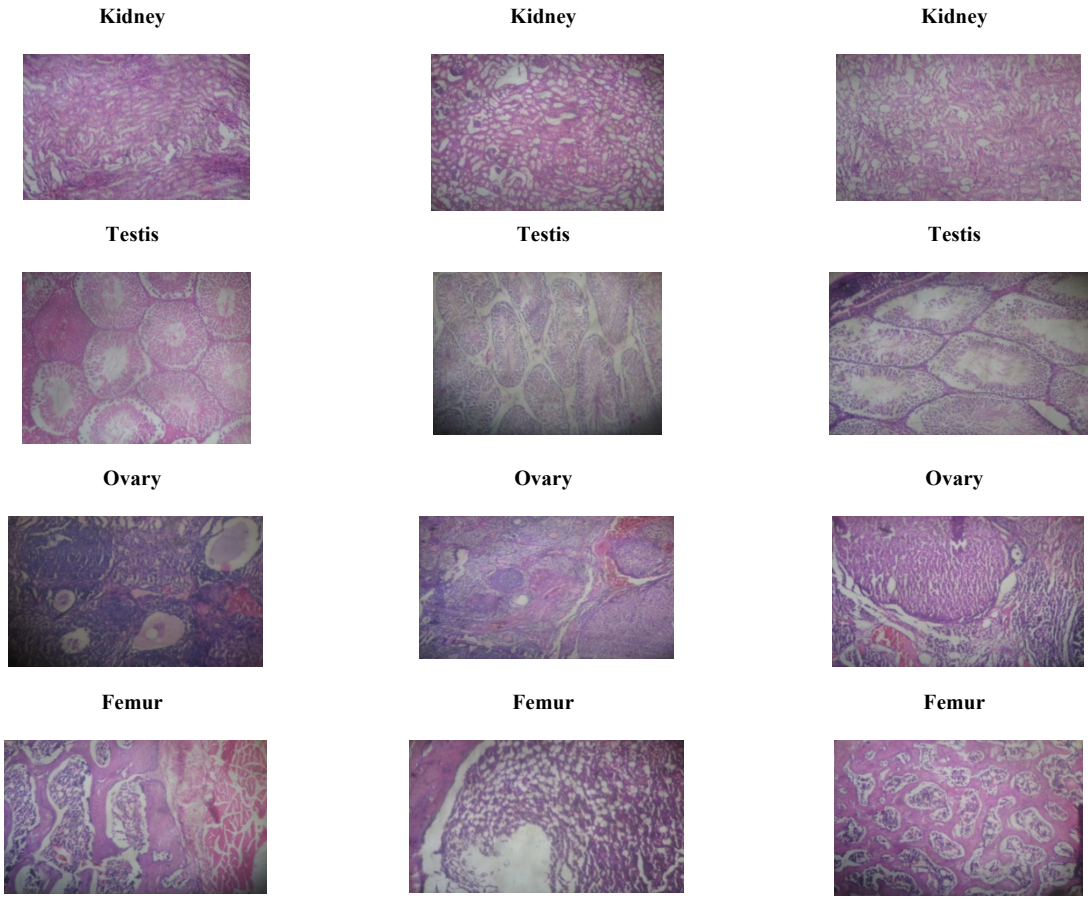

Figure 1: Histopathological results of various organs of control and 28 days Gandhaga baga parpam treated wistar albino rats

Heart, lung, liver, stomach, pancreas, small intestine, spleen, kidney, testis, ovary and femur are normal in control group. In Group II liver showed chronic venous congestion and other studied organs are normal. In Group III liver showed chronic venous congestion, periportal lymphocytic infiltration, Stomach showed hyperplasic of gastric mucous with focal surface ulceration and other organs are normal (Figure 1)

DISCUSSION

In acute toxicity study the oral administration of the Gandhaga baga Parpam in doses from 1000 to 2000 mg/kg produced significant behavioral changes and various sign of toxicity like severe itching, breathing, cutaneous effects, sensory nervous system responses in male and female rats (Table 1). These effects were observed during the experimental period (24 h). During 24h of the experiment, deaths occurred in 1000 mg/kg treated groups. These results showed that in single dose, there are adverse effects of Gandhag baga Parpam, indicating that the median lethal dose (LD_{50}) is 1000 mg/kg for rats.

The results of 28 days repeated oral toxicity study showed no behavioral changes and mortality during study period. In test drug treated animals body weight was significantly decreased at III and IV weeks (Table 2), Food and water intake were significantly decreased II, III and IV weeks when compared with I week (Table 3 and 4). Hematological results showed significant changes in Hb in Group I, Group II when compared with control group. Significant changes in SGOT, SGPT, ALP and Kidney function test and lipid profile were observed in Group I and Group II when compared with control group (Table 6-8). Histopathology results revealed mild changes in liver of Group II, Group III and Stomach of Group III and no abnormality detected in other vital organs. As per above findings No observed adverse effect level and Low observed adverse effect level of Gandhaga baga parpam was 100 mg/kg.bwt and 200 mg/kg.bwt.in rats.

CONCLUSION

Acute and 28 days repeated oral toxicity study of Gandhaga baga parpam was carried out in wistar albino rats. The LD50 of Gandhaga baga parpam was 1000 mg/kg b. wt found out by acute toxicity study. The 28 days repeated oral toxicity study revealed No observed adverse effect level and Low observed adverse effect level of Gandhaga baga parpam was 100 mg/kg. bwt and 200 mg/kg.bwt. Further pharmacological studies are required to strengthen the therapeutic effect of Gandhaga baga parpam

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