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RESEARCH ARTICLE

ACUTE AND 28 DAYS REPEATED ORAL TOXICITY STUDY OF SIDDHA DRUG GANDHAGA CHOORNAM ON WISTAR ALBINO RATS

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ABSTRACT

Diabetes Mellitus is a common metabolic disorder seen in all age groups. *Gandhaga chooranam* (GC) has been employed as a traditional remedy for Diabetes mellitus which is a herbo mineral formulation. As a mandate, steps were taken to evaluate safety profile of GC in rats following OECD guidelines. Acute toxicity studies, were done on female wistar albino rats as per OECD guidelines 423 and 28 days repeated oral toxicity study was done on wistar albino rats of both sex as per OECD guidelines 407.Acute oral toxicity study of GC revealed no mortality at the dosage of 2000 mg/kg body weight and the median lethal dosage of GC is estimated to be above 2000 mg/kg body weight. Repeated oral toxicity study of GC does not exhibit mortality at the high dosage of 400 mg/kg body weight given up to the period of 56 days including 28 days of drug administered. At the end of 28 days no specific changes are observed in hematological, hepatic, renal and other biochemical parameters. No gross morphological and histological changes were observed in the organs. The GC was found to be safe in animals. No toxic effect was observed upto 400 mg/kg of *Gandhaga chooranam* in acute and sub-acute toxicity studies. The above studies recommend that GC is a safest drug under intended human adult dosage (1000 mg) as illustrated in the literature.

INTRODUCTION

Diabetes mellitus is a fastest growing metabolic disorder in the world and major cause of morbidity in developing countries (King *et al.,* 1998). It is characterised by hyperglycaemia with or without glycosuria, resulting from an absolute or relative deficiency of insulin. It is due to the resistance to the action of insulin either due to a receptor/post receptor defect or an imbalance between insulin and its counter regulatory hormones. The prevalence of diabetes is likely to double during the next few years. By the year 2025, the number of people suffering from diabetes in the world is estimated to be around 350 million, of which 70 million will be in India. There are two main types of diabetes labeled as Type I diabetes (Insulin dependant diabetes mellitus) and Type II diabetes (Non-Insulin dependant diabetes mellitus) (Krishna 2008). In the recent years, complementary and alternative medicine (CAM) has upsurge globally for the treatment and prevention of many ailments which are non communicable and chronic in nature (Astin 1998). The interventional drug *Gandhaga chooranam* (GC) is a compound Siddha formulation containing Gandhagam (Purified Sulphur), Seenthil sarkarai (*Tinospora cordifolia*) and Parangipattai (*Smilax china*) has been quoted in Aviyalikkum Amuthamurai Churukkam for the treatment of Madhumegam (Kandhasamy Mudaliar,1975). In Siddha literature Purified Gandhagam is indicated for urinary disorders, venereal diseases, skin problems and general debility (Thiyagarajan 2006). Seenthil Sarkarai (Starch of Tinospora stem) is useful in urinary disorders, mega noigal and thirst in diabetes (Murugesa Mudaliar 1936). Parangipattai is used in thirst,skin diseases, carbuncles and diabetes (Murugesa Mudaliar 1936). But a clear picture of toxicokinetics of GC has not been studied earlier. So this article ventured to evaluate the acute and sub acute toxicity of herbo-mineral formulation *Gandhaga chooranam* in laboratory animals.

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MATERIALS AND METHODS

Preparation of *Gandhaga Chooranam*

a. **Ingredients:** The Siddha medicine GC has ingredients such as Purified Gandhagam-140 g, Seenthil Sarkarai (*Tinospora cordifolia*)-70 g and Parangipattai (*Smilax china)*-140 g.
b. **Procedure:** All ingredients are powdered, mixed and prepared as Chooranam (Kandhasamy Mudaliar 1975).

Aim: Aim of the study is to evaluate the acute and sub-acute toxicity of the Siddha drug 'Gandhaga Chooranam'.

Animals

Wistar albino rats of either sex (8-12 weeks old), were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai. The animals were used with the approval of the Instituitional Animal Ethical Committee (IAEC) obtained from Sairam Advanced centre for Research, Chennai on 26.03.2013 bearing no.1545/po/a11/ CPCSEA/1-2/2013. The animals were fed with standard pellet diet (Sai Meera foods, Bangalore) and kept under standard environmental condition (23±2ºC) temperature, standard light cycle (12 hr light, 12 hr dark) and water ad libitum. Animal welfare guidelines were observed during the maintainence period and experimentation. The animals were randomly selected, as control and different treatment groups, three animals for each step. and were kept in cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute toxicity study-OECD-423 guidelines (Schlede *et al.*, 1994; Schlede *et al.*, 1992)

Acute oral toxicity test for Gandhaga chooranam was carried out as per OECD guidelines 423. Care was taken to ensure that animals are available in appropriate size and age range for the entire study. GC was suspended in 2% CMC administered to female rats in single oral dose by gavage using a feeding needle. Following the period of fasting, animals were weighed and test substance was administered. After this food is withheld for a further 3-4 hrs. After dosing, animals were observed atleast once during first 30 minutes, periodically during the first 24 hrs., with special attention during the first 4 hrs, and daily thereafter for a total of 14 days to observe any behavioural changes, physiological activities and mortality. The substance is tested using a stepwise procedure, as compound related mortality of animals determines the next step. Observations include changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor and behavior pattern and time of onset and length of recovery time were systematically recorded.

28 days Repeated oral toxicity study-OECD guidelines 407 (OECD Adopted on 12 May 1981 and Updated on 27 July 1995)

In sub-acute toxicity study forty rats (5+5) of either sex were divided into four groups of 10 rats each. Group I served as normal control was administered with CMC with distilled water (p.o), while Groups II, III and IV were administered daily with *Gandhaga chooranam* for 28 days at a dose of 40,200 and 400 mg/kg body weight (p.o) respectively.

The animals were observed daily for behavioural changes and other signs of sub-acute toxicity [Table 1] The weight of each rat was recorded on day 0 and weekly throughout the course of the study [Table 2], Food and water consumption per rat was calculated [Table 3 & 4]. On the 29th day, animals were fasted for 18 hrs, slightly anesthetized with ether and blood samples were collected from retro-orbital plexus into two tubes.

Haematological and Bio chemical analysis

The blood collected in one tube added with EDTA and heparinized for immediate analysis of Haematalogical parameters (red blood cell count, total white blood cell count, differential white blood cell count, platelet count and haemoglobin) by semi auto analyzer [Table 5]. Blood collected in other tube without any anticoagulant was centrifuged at 4000 rpm at 4ºC for 10 minutes to obtain serum and stored at 20º C and analysed for biochemical parameters like protein, bilirubin, urea, creatinine, triglycerides, cholesterol and glucose using standard methods and SGOT, SGPT were estimated as per calorimetric procedure [Table 6,7,8 & 9].

Necropsy

All rats were sacrificed on day 29. The positions, shapes, size and colour of organs were evaluated. The weight of organs like brain, heart, lung, stomach, liver, spleen, kidney, ovary and testes were recorded and relative organ weight was then calculated and preserved in 10% formalin for Histopathological assessment [Table-10]. The tissues were embedded in paraffin and stained with haematoxylin and eosin and examined microscopically [Panel1-4].

Statistical analysis

Values were represented as mean ± SEM. Data were analyzed using one way Anova following Dunnet 't' test using a computer software programme INSAT V3 version. P values < 0.05 were considered significant.

RESULTS

Panel 1: Light photomicrography of liver of a control rat
Figure A – Control
Figure B – Treated on high dose, hepatic congestion and hemorrhage is seen in hepatocytes

Panel 2: Light photomicrography of Spleen of a control rat
Figure A – Control
Figure B – Treated on high dose, medullary congestion is seen in trabeculae, capsule.

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

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 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.

Table 2. Body weight (g) changes of albino rats exposed to *Gandhaga chooranam* for 28 days

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	122.37±3.21	124.14±4.09	118.21±2.17	127.21±5.11	133.32±1.89
40	125.31±2.32	125.32 ±2.14	126.29 ±3.81	129.24 ±2.96	132.19±4.25
200	127.19 ±3.64	129.39 ±6.14	131±2.96	132.48 ±4.23	134.14±5.32
400	127.38±5.42	128.87±2.86	131.57 ±7.52	131.62±4.32	132.27±5.23

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

Table 3. Food (g/day) intake of albino rats exposed to Gandhaga chooranam for 28 days

Dose (mg/kg/day)	Days(g/rats)				
	1	7	14	21	28
Control	38.25±3.10	36.18±2.78	39.36±2.10	36.14±2.80	39.20±2.12
40	38.42 ±1.96	38.22 ±1.79	38.72 ±2.47	38.45 ±2.31	39.21 ±1.87
200	39.92 ±2.21	39.51 ±2.65	39.31 ±2.11	40.12 ±1.99	41.12 ±2.11
400	41.42±3.01	42.12 ±2.42	42.75 ±1.97	42.85 ±2.78	43.42 ±2.97

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10

Table 4. Water (ml/day) intake of albino rats exposed to Gandhaga chooranam for 28 days

Dose (mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	44.24±3.00	43.32 ±3.22	45.21 ±3.14	43.32 ±2.54	42.10±2.96
40	44.24 ±2.34	46.32 ±2.42	47.31 ±1.78	49.52 ±2.74	50.42 ±2.68
200	49.31 ±2.14	48.98 ±2.13	48.36 ±3.12	48.14 ±2.34	48.24 ±2.14
400	48.23 ±2.14	48.32 ±2.14	48.94 ±1.96	49.34 ±2.14	50.24 ±1.75

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10.

Table 5. Effect of Gandhaga chooranam on Haematological parameters after 28 days

Parameter	Control	40 mg/kg	200 mg/kg	400 mg/kg
RBC (mm^3)	7.51±0.16	6.25±0.11	6.48 ±0.22	6.87±0.31
HB (%)	15.60 ±0.19	15.21 ±1.23	15.35 ±2.14	15.35 ±1.23
Leukocyte ($x10^6$/mL)	10124±126.51	10157±142.03	10307±196.14	10338±214.21
Platelets/ul	1335±17.32	1310±22.14	1321±32.12	1342±42.31
DLC (%)				
N	4.38 ±1.42	4.48 ±1.24	5.50 ±3.12	5.22 ±2.31
L	91.12±4.12	91.15 ±2.14	91.31 ±2.31	91.21 ±1.98
M	2.11±1.23	2.24±0.80	2.42 ±1.45	2.75±1.23
E	1.12±0.22	1.48 ±1.23	1.58±1.34	1.65 ±2.14
B	0	0	0	0

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=10

Table 6. Effect of *Gandhaga choranam* Hepatic parameters

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Total Bilirubin (mg/dL)	0.205±1.01	0.206±012	0.312±0.23*	0.416 ±2.31*
SGOT (U/L)	166.44±3.24	164.78±2.52	162.42±3.45	159.14±2.12
SGPT(U/L)	45.4±2.14	55.25 ±2.34	74.78 ±1.98*	85.43 ±2.34*
Total Protein(g/dl)	10.62±1.30	9.42±0.27	10.11±0.46	10.16±0.30

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=10.

Table 7. Effect of *Gandhaga choranam* on Lipid profile

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Total cholestrol(mg/dL)	41.24±1.35	42.19 ±2.10	43.13 ±1.96	44.15±2.31
HDL(mg/dL)	12.40 ±1.45	12.50 ±2.14	12.75 ±2.10	13.19±1.23
LDL(mg/dL)	35.14±1.88	37.14±2.47	38.18±2.88	42.11±3.18
VLDL(mg/dl)	16.45 ±2.46	14.10±1.13	16.36 ±1.66	16.24 ±2.10
Triglycerides (mg/dl)	80.15 ±3.21	81.22±1.35	82.25 ±1.14	83.12 ±2.45
TC/HDL ratio (g/dl)	3.24 ±2.47	3.34±1.21	3.49±2.07	3.44±2.5
Blood glucose(mg/dl)	110.16±8.62	120.0±3.33	122.37±4.12	123.4±1.98

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=10.

Table 8. Effect of *Gandhaga choranam on* Renal parameters

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Urea(mg/dL)	64.24 ±3.11	68.13 ±1.42	83.32 ±2.14*	94.12 ±2.31*
Creatinine (mg/dL)	0.82±0.16	1.23±0.14	1.89±1.32*	2.01±1.21*
Uric acid (mg/dL)	1.6±0.21	1.56±0.26	1.6±0.22	1.6±0.23
Na m.mol	138.12±3.14	137.4±3.41	138.12±3.14	139.18±2.01
K m.mol	20.50±2.34	19.51±2.18	20.10±2.28	20.23±2.20
Cl m.mol	99.24±2.11	98.12 ±1.47	100.22 ±1.69	103.20±2.31*

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=10.

Table 9. Effect of *Gandhaga choornam* on Urine parameters

Parameters	Control	40 mg/kg	200 mg/kg	400 mg/kg
Colour	Yellow	Yellow	Light brown	Dark brown
Transparency	Clear	Clear	Haziness	Cloudiness
Specific gravity	1.010	1.007	1.024	1.1
pH	7.2	7.8	9.2	9.6
Protein	Nil	Nil	Trace	1+
Glucose	Nil	Nil	Nil	Nil
Ketones	Nil	Nil	Nil	Nil
Bile salts	Nil	Nil	Nil	+
Bile pigments	Nil	Nil	Nil	+
Urobilinogen	Normal	Normal	Normal	Increased
RBCs	Nil	0-1cells/HPF	2 cells/HPF	6 cells/HPF
Pus cells	0-cells/HPF	0-cells/HPF	0-cell/HPF	1-cell/HPF
Epithelial cells	Nil	Nil	ocasional	+
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	+
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=10.

Table 10. Effect of *Gandhaga choranam* on Organ weight

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Liver (g)	5.24±0.14	4.23±0.22	4.45±0.21	4.75±0.20
Heart (g)	0.70±0.05	0.66±0.02	0.67±0.02	0.65±0.05
Lung (g)	1.78±0.25	1.74 ±0.10	1.53 ±0.21	1.45 ±0.22
Spleen (g)	0.74 ±0.07	0.66±0.04	0.62 ±0.05	0.69±0.05
Ovary (g)	1.91±0.14	1.66±0.15	1.86±0.12	1.67±0.18
Testes (g)	1.40±0.12	1.43±0.19	1.41±0.12	1.42±0.12
Brain (g)	1.43±0.18	1.44±0.18	1.42±0.17	1.44±0.16
Kidney (g)	0.70±0.05	0.72±0.04	0.71±0.04	0.72±0.05
Stomach (g)	1.23±0.10	1.23±0.12	1.12±0.20	1.30±0.17

Values are mean of 10 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01 *vs control* N=10.

Panel 3: Light photomicrography of Heart of a control rat

Figure A – Control
Figure B – Treated on high dose, abnormality is seen in nuclei of Myocytes, myocardium

Panel 4: Light photomicrography of Kidney of a control rat

Figure A – Control
Figure B – Treated on high dose, significant abnormality is seen in glomeruli, Bowman's capsule, capillaries.

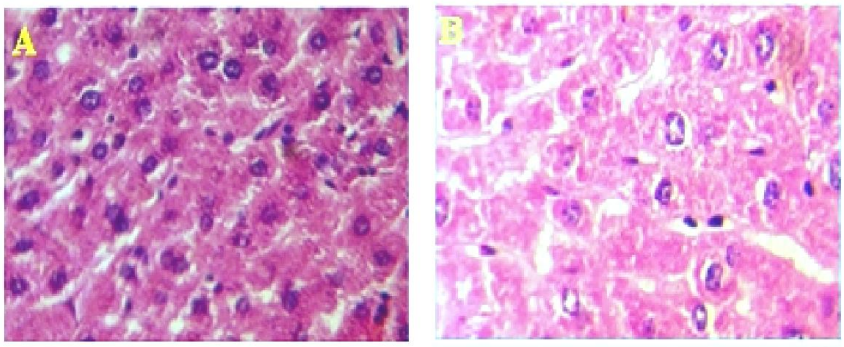
DISCUSSION

Acute oral toxicity

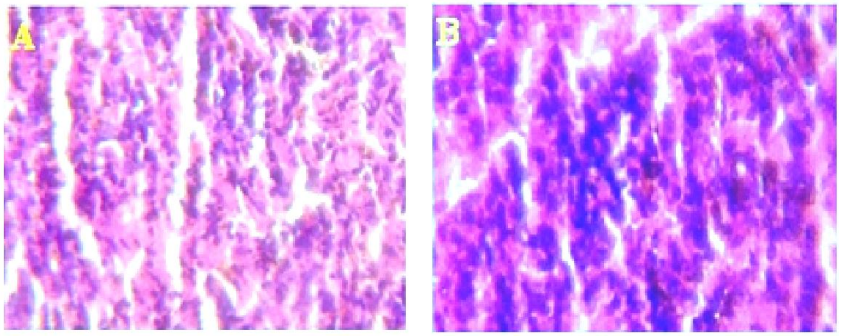
The results showed that no changes in behavior pattern except grooming and gripping, no mortality and abnormal signs were observed at the dose of 2000 mg/kg bodyweight. The mean lethal dose should be above 2000mg/kg body weight which comes under unclassified.

Sub-acute toxicity

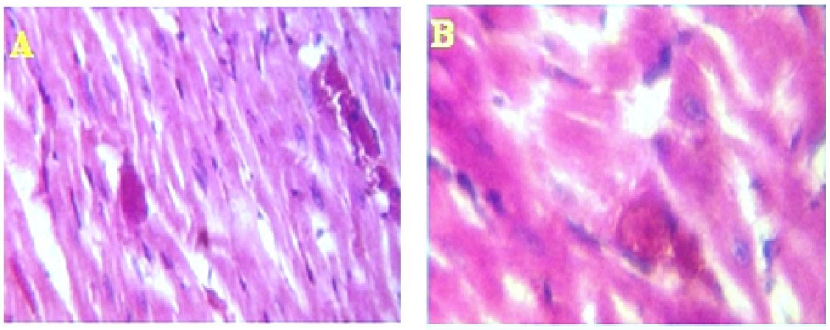
All the animals from control all treated dose groups upto 400mg/kg survived throughout the dosing period of 28 days. No significant changes in behavior pattern was observed. No significant weight differences in bodyweight were observed in treated and control groups. Food and water consumption was comparable throughout the dosing period of 28 days. The haematological parameters were within normal limits in control and treated groups even at higher dose. Results of biochemical parameters showed that significant changes in values of urea, creatinine, SGPT, bilirubin at dose of 400mg/kg (P<0.005) when compared with those of respective controls. But these values were within normal range for rats. Total cholesterol and LDL level were elevated in at dose of 400 mg/kg but these were within normal limits for rats. Urine analysis, conducted revealed no abnormality attributable to the treatment. Organ



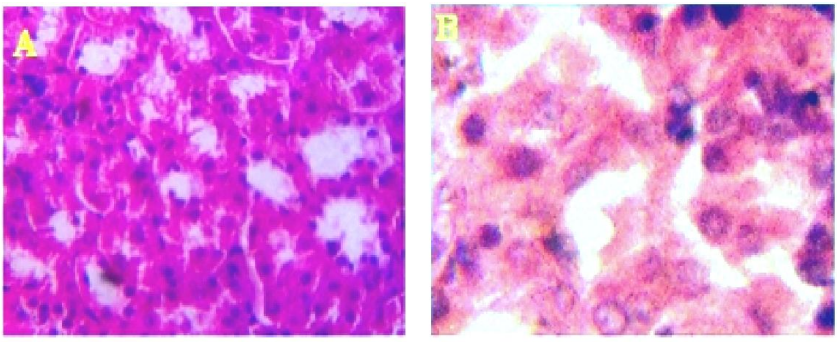
Panel 1: Light photomicrography of liver of a control rat



Panel 2: Light photomicrography of Spleen of a control rat



Panel 3: Light photomicrography of Heart of a control rat



Panel 4: Light photomicrography of Kidney of a control rat

weight of animals shows slight decrease in weight of liver in treated group but it was not statistically significant. Gross pathological examination revealed no abnormality. Histopathological examination did not reveal any abnormal macroscopic changes. Microscopically changes were observed in organs only at 400mg/kg body weight which is higher than human intended dose.

Conclusion

Based on these findings, no toxic effects were observed upto 200mg/kg of *Gandhaga Chooranam* on oral route over a period of 28 days.So it can be concluded that *Gandhaga Chooranam* can be used as a therapeutic agent in treating reported diseases effectively with dosage recommendations upto 400 mg/kg body weight p.o.

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