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RESEARCH ARTICLE

ACUTE AND SUB ACUTE TOXICITY STUDIES OF GANDHAGA CHUNNAM ON SWISS ALBINO RATS

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ABSTRACT

Psoriasis is a chronic, non- infectious skin disease affects both sexes. Gandhaga Chunnam (GC) has been employed as a traditional remedy for psoriasis which is a siddha herbo-mineral formulation contains gandhagam (sulphur) in its calcinated form. GC is further evaluated for toxicological effects. Acute and sub acute toxicity studies with GC were carried out on Swiss albinorats. During acute toxicity study there were no any adverse effects found in the general behavior and no mortality at any dose level given(2000 mg/kg/b.wt). In sub acute toxicity study in the dose of(200,400 mg/kg/b.wt) didn't cause any changes in haematological & bio –chemical parameters like leucocyte count, free fatty acid, plasma and urine, creatinine level. Further histo-pathological examination of vital organs should normal suggesting no morphological disturbances. It can be considered that GC is safe and non-toxic.

INTRODUCTION

Psoriasis is an auto immune disease characterized by well defined, slightly raised, dry erythematous macules with white silvery scales with typical extensor in distribution. (Behl 1st Edition 1962, Reprint 2007, 2009, 2011. In Indian systems of medicine most practitioners formulate and dispense there own recipes. GC herbo-mineral formulation mainly consists of gandhagam. Honey was used as an vehicle to promote there action. Gandhagam (sulphur) has been reported to have Astringent action (Thiyagarajan *et al.,* 1952). Sulphur can maximize skin health and it act as essential dietary mineral for both skin health and overall wellness of the body. Deficiency of this mineral in our body leads to progression of inflammatory skin diseases (Pub med: The use of Sulphur for Skincare July 23rd, 2010 / by Sarah Terry. www.livestrong.com / articles / 18286) In siddha literature Gandhagam is indicated for skin disorders, veneral diseases, urinary disorders and general debility (Thiyagarajan 2006). Coconut oil is used as a skin moisturiser (Murugesa mudhaliyar 1936). Egg white has an emulsent action (Gunapaadam thathu and jeeva vaguppu). But the proper toxicity of GC has not been studied earlier. So this research article is useful to evaluate the acute and sub acute toxicity of herbo mineral formulation GC in laboratory animals.

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MATERIALS AND METHODS

Gandhaga chunnam has been prepared by the following method as per the text book (Anooboga vaidhya navaneetham 3rd Edition, 1st Print 1995, 3rd Print 2006)

Ingredients

1.	Gandhagam(Sulphur)	–	1400gm (40 palam)
2.	Coconut oil	–	2800gm (80 palam)
3.	Egg white	–	Quantity sufficient.
4.	Cucumber	–	4 in number

Purification of drugs

Take large pieces of nellikkai Gandhagam (Sulphur) and embedded it in 3 to 4 long cucumber. Wrap the cucumber with thread. Heat the coconut oil in a pot and put that wrapped cucumber until the threads become charred. Allow it to cool and stored in a container.

Preparation method

Then the purified Gandhagam (Sulphur) is grounded with egg white for 3 hour (1 saamam) and made into pills of thetrankottai seed size (500mg) and dried in sunshade. After that the pills are placed inside the egg shell and it is covered by

3 layers of mud pasted cloth (seelaiman) and are subjected for incineration (pudam) with 15 palam earu (cow dung cake – 525gm). Then the content is cooled and triturated in a stone mortor and then stored.

Dose: 65mg twice a day.
Adjuvant: Honey.
Duration: 48 days.
Aim: To evaluate the acute and sub acute toxicity of the siddha drug 'Gandhaga chunnam'.

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

Acute toxicity study for GC was carried out as per OECD guidelines423. Healthy female rat weighing 200-250 gms were divide into three groups. The animals were housed under standard conditions and room temperature was controlled. All animals were fed with standard rat pelleted diet. Studies carried out at 3 female rat under fasting condition. Signs of toxicity was observed for every one hour for 1st 24 hours and every day for about 14 days to observe any behavioural changes, physiological activities and mortality. Observations include changes in skin colour, eyes, mucus membrane, respiratory, circulatory, autonomic nervous system were systematically recorded. Study has got approval from the Institutional animal ethics committee (IAEC)(Approval No: IAEC/XXXIX/08/ CLBMCP/2013). GC was administered orally at doses of (50,100, 250,500,1000,2000 mg/kg/b.wt). No signs of toxicity was observed.

Sub Acute Toxicity study: (OECD guidelines 407) (Schlede *et al.,* 1992)

In sub acute toxicity study swiss albino rats of either sex weighing 220-250 gms were divided into three groups of 6 animals each (three male and three female) and were housed under the same conditions as described above. GC were administered for 28 days at doses of 200 and 400 mg/kg/b.wt respectively. The animals were observed daily for behavioural changes and other signs of sub acute toxicity. Toxic manifestations and mortality were monitored daily and body weight changes are recorded every 7 days till the end of study. The weight of each rat was recorded on day 0 and weekly thought the course of the study. Food and water consumption per rat was calculated. On 29th day, animals were fasted for 18 hours, slightly anesthetized with ether and blood samples were collected from retro-orbital plexes.

Clinical parameters checked

Group	Day
Body Weight	Normal
Assessments of posture	Normal
Signs of convulsion Limb paralysis	Normal
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant color change
Piloerection	Not observed
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle grip ness	Normal
Rearing	Mild
Urination	Normal

Effect of GC on Haematological analysis

The blood collected in one test tube and then added with EDTA and add heparin for immediate analysis of Haematological parameters (RBC count, total WBC count, differential white blood cell count, platelet count and haemoglobin) by semi auto analyser method. Blood collected in another tube without heparin was centrifuged at 4000 rpm at4^0 c for 10 minutes to obtain serum and then stored at 20^0 c for analysing of bio chemical parameters like creatinine, triglycerides, cholesterol and glucose by using standard methods.

RESULTS

Acute Toxicity study

There were no mortality and no behavioral changes, toxicity observed after oral administration of GC upto the dose level 2000 mg/kg/b.wt in rats.

Sub acute Toxicity study

There was no significant difference between the control and GC treated groups. No lethality was recorded for any dose upto the maximum of 400 mg/kg during the 28 day period of testing.

Statistical analysis

Effect of GC in animal model was effective and highly significant.

Grouping	Total RBC Count($x10^6$ µ l)	Total WBC Count($x10^3$ µ l)	Platelet count($x10^3$ µ l)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood Sugar ® (mg/dl)	BUN (mg/dl)
Control									
Mean	7.667	7.333	567.7	45.5	56.17	21.67	35	76.5	16.5
SD	1.506	0.8165	5.61	3.271	2.317	4.227	4.49	3.146	2.811
SE	0.6146	0.3333	2..29	1.335	0.9458	1.726	1.751	1.285	1.1147
Low Dose									
Mean	8.417	9.35	570.5	47.83	58.67	24.17	41.5	74.5	13.58
SD	1.009	1.193	2.074	2.563	4.082	4.708	1.975	3.834	1.855
SE	0.4118	0.487	0.8466	1.046	1.667	1.922	0.8062	1.565	0.7574
High Dose									
Mean	8.583	6.883	564.2	42.67	55.5	20	45.67	71.33	14
SD	0.7859	1.234	4.916	3.777	3.619	2.366	3.882	3.615	3.347
SE	0.3208	0.5036	2.007	1.542	1.478	0.966	1.585	1.476	1.366

Effect of GC on lipid profile

Grouping	Serum creatinine (mg/dl)	Serum Total Colorestrol (mg/dl)	SerumTGL Level (mg/dl)	SerumHDL Level (mg/dl)	Serum LDL Level (mg/dl)	Serum VLDl (mg/dl)
Control						
Mean	0.8167	97.67	46.33	26.7	45.5	35.5
SD	0.07528	3.077	3.327	2.733	3.507	3.728
SE	0.03073	1.256	1.358	1.116	1.432	1.522
Low Dose						
Mean	0.8167	107.2	49.67	26.83	46	34.5
SD	0.2714	2.563	4.131	3.251	4.336	3.45
SE	0.1108	1.046	1.687	1.327	1.77	1.408
High Dose						
Mean	0.5333	102.5	41.83	32.5	42.33	35
SD	0.1633	4.183	2.848	2.51	3.502	4.05
SE	0.06667	1.708	1.167	1.025	1.43	1.653

Effect of GC on Body weight and Food consumption

Grouping	Food (g/day/rat)	Body weight (g)
CONTROL		
MEAN	20.17	231
SD	1.329	9.839
SE	0.5426	4.017
LOW DOSE	Food (g/day/rat)	Body weight (g)
MEAN	28.5	231.3
SD	3.728	3.615
SE	1.522	1.476
HIGH DOSE	Food (g/day/rat)	Body weight (g)
MEAN	21.67	225.8
SD	2.944	4.834
SE	1.202	1.973

Effect of GC on Mortality rate of the study animals

Treatment	Mortality observed for the duration of 1- 28 days
GROUP I – CONTROL	NIL
GROUP II- LOW DOSE	NIL
GROUP III- HIGH DOSE	NIL

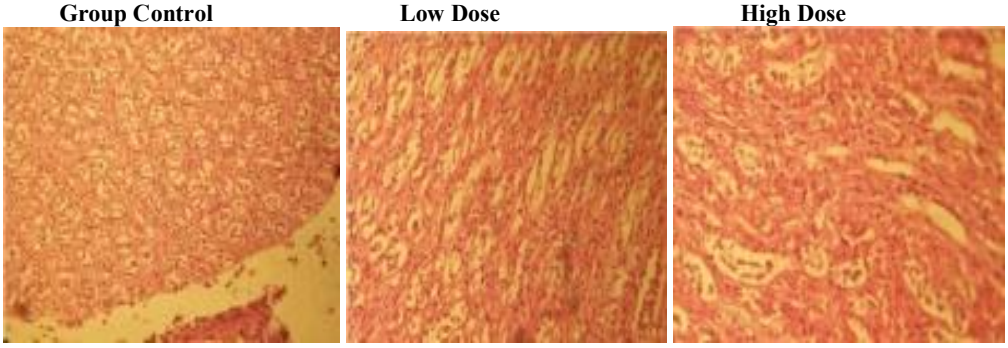
Effect of GC on organ morphology

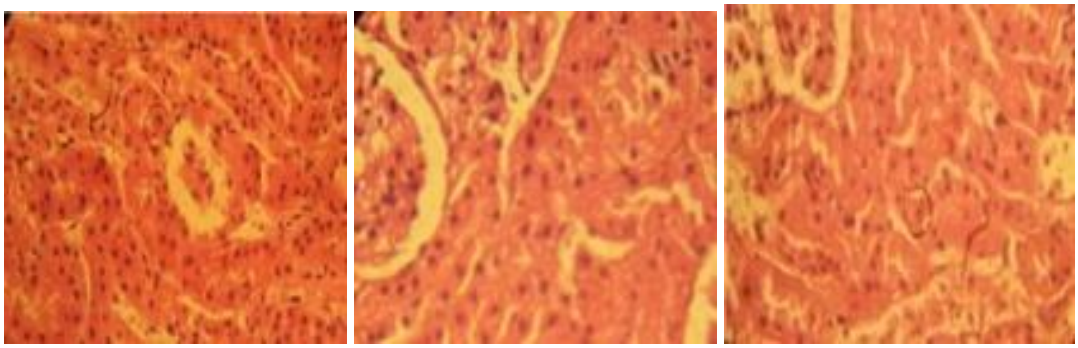
Grouping	Kidney	Liver	Heart	lungs	Spleen	pancreas	Brain	Ovaries	Testes
GROUP I CONTROL	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
GROUP II LOW DOSE	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
GROUP III HIGH DOSE	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

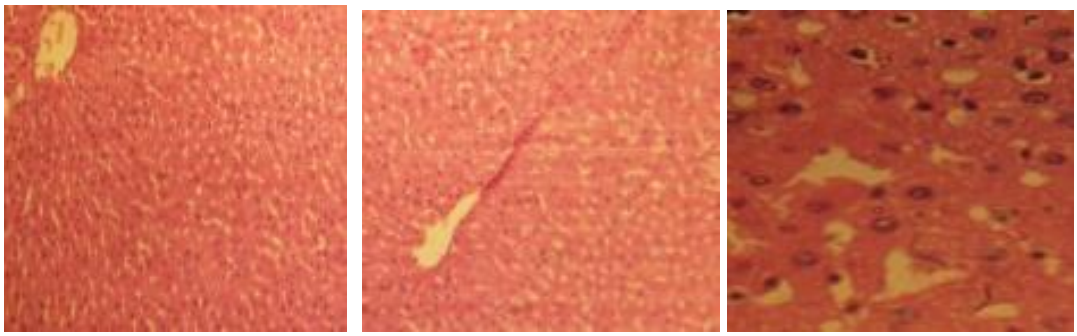
Histo pathological analysis of Sub-Acute toxicity study

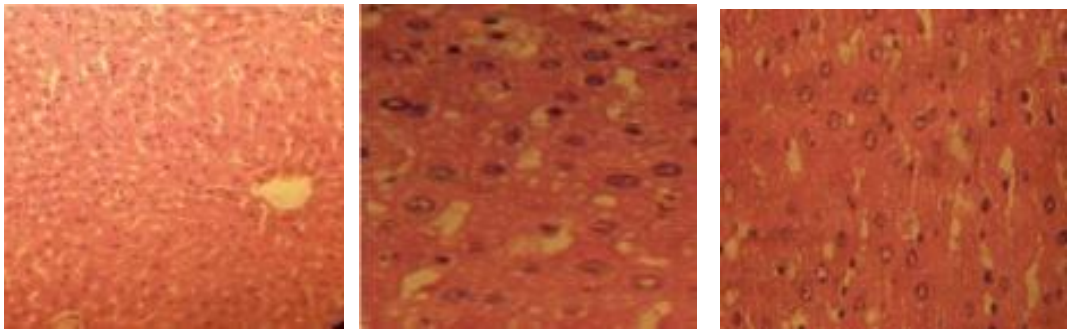
Kidney Magnification: Low Power 10X

Group Control **Low Dose** **High Dose**

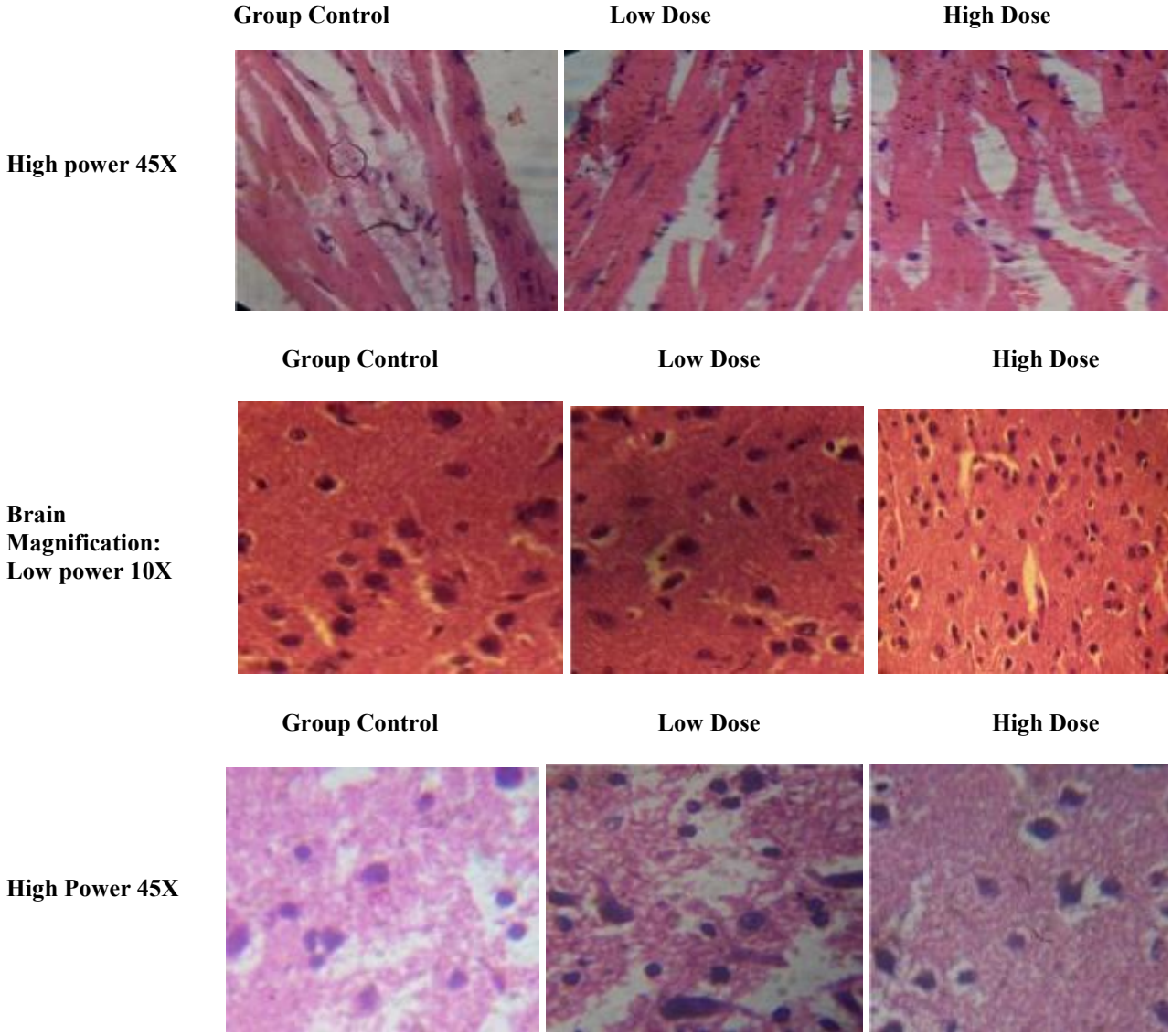


	Group Control	Low Dose	High Dose
High power 45X			

	Group Control	Low Dose	High Dose
Group Control Low Dose High Dose			

	Group Control	Low Dose	High Dose
High Power 45X			

	Group Control	Low Dose	High Dose
Heart Magnification: Low Power 10X			



PATHOLOGIST REPORT

Sample	Observation
Kidney	Pattern of nephrons and arteries appears normal , no Sevier abnormalities in all three groups
Heart	Nucleus appears dense and intense and myocardial fiber length appears normal.
Liver	No signs of necrosis or cirrhosis were observed in all the groups. Lumen of hepatic veins appears normal.
Brain	Neuronal integrity appears normal in all the groups and also no considerable observation of signs of edema or degeneration

Software: spss17 version
Test: Paired t test
Confidence Interval: 95%
Correlation coefficient (r): 0.736
Mean difference ± SD: 17.07 ± 8.41
P Value (2 tailed): p<0.01.

Inference

Since the P value is highly significant (<0.01). Hence it is concluded that the GC was effective and significant.

DISCUSSION

In Acute toxicity study there was no any mortality observed upto the maximum level of 2000 mg/kg/b.wt of GC administered orally, which the single high dose recommended by OECD guidelines – 423[5] for testing acute toxicity. In that GC doesn't cause any acute toxicity. The changes in body weight have been used as an indicator of adverse effect of GC and chemicals (Ecobichon 1997). Since there were no changes in animal behaviour, body 7 organ weights at all doses of treated rats when compared to the control group. The present results reveal that at the oral doses administered GC is non-

toxic. Histo pathological studies of skeletal organs (Heart, Liver, Kidney, and Brain) from both treated and control animals shows normal architecture, suggesting no detrimental changes and morphological disturbances caused due to the administration of GC for 28 days.

Conclusion

GC can be considered safe and didn't cause either any lethality or adverse changes with general behavior of rats in aute toxicity study upto the dose of 2000 mg/kg b.wt and also there was no observed detrimental effects caused by GC (upto 400 mg/kg b.wt) in sub acute study rat model. Further the above results substantiate the beneficial and enhanced pharmacological effects of the herbo mineral formulation GC.

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