International Journal of Research and Review
Vol.7; Issue: 3; March 2020
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Toxicological Profiling of a *Siddha* Formulation *Ghendhiyathi Uppu* in Experimental Rodents

Thilagavathi.R[1], Kavitha.T[2], Esaivani.S[2], Susila.R[2]

[1]Research Officer (Siddha), Siddha Clinical Research Unit, Bengaluru
[2]Research Officer (Siddha), Siddha Central Research Institute, Arumbakkam, Chennai

Corresponding Author: Thilagavathi.R

ABSTRACT

Ghendhiyathi Uppu (GU) is a *Siddha* formulation indicated in literature for Pleurisy. This study was aimed to evaluate the Acute and Chronic oral toxicity study of *Ghendhiyathi Uppu (GU)* in albino rats to evaluate the traditional claims for the safety of the test drug. It was observed that the formulation was found to be non-toxic at dosage of 1600mg/100gm body weight of the animal in albino rats and so it can be classified under category-4 of globally harmonized system of classification and labelling of chemicals in Acute toxicity study and the drug produces mild histopathological changes during long term administration at the dose of 200mg/100gm of body weight, but not in the lower dose of 100mg/100gm of body weight of the animal. The biochemical and haematological parameters were analysed and the Histopathological study was performed after 90 days repeated oral toxicity. Through this study the *Siddha* formulation *Ghendhiyathi Uppu (GU)* can be evidently accepted to be nontoxic to the humanity at the lower dose of 100mg/100gm of the body weight.

Keywords: *Ghendhiyathi Uppu (GU), Siddha*, Acute toxicity, Chronic toxicity, Traditional medicine.

INTRODUCTION

The *Siddha* system of medicine is one of the age-old systems that owes its origin to the thoughts and practices of Tamil sages who belonged to Southern parts of India called *Siddhars*. The *Siddha* literature has a vast collection of herbal and mineral drugs for the treatment of various common ailments of humans. [1,2] Siddhars successfully used their extensive knowledge of iatrochemistry, minerals, metals and plants from time immemorial. Processes like calcination of mercury, minerals, metals and the preparation of a super salt- 'Muppu', animated mercury pills with high potency possessing marvellous properties of transmuting metals and capable of rejuvenating the entire human system bear ample testimony to the fact that even in ancient times, when knowledge in chemical technology was not advanced or developed, Siddhars had an unparalleled knowledge of medicine preparation. The above processes are unique to Siddha system of medicine. The preparations in which Siddhars specialized are metallic preparations *(chunnam)* which are alkaline; waxy preparations *(mezhugu)*; and preparations which are impervious to the water and flame *(kattu)*. These preparations have a longer shelf life and their potency and efficacy improve with time. They do wonders in very little dosage [3] Though these formulations have been time tested for their safety and efficacy, most of them have not been scientifically validated. *Ghendhiyathi Uppu (GU) is* the Siddha formulation with the combination of Nellikkai Ghendhagam (Sulphur) and Uppu (Sodium Chloride) that has been mentioned in *Sarabenthirar Vaidya muraigal a* Siddha literature for the treatment of pleurisy. Through this research an effort has been made to explore acute and chronic oral toxicity of the Siddha formulation

Ghendhiyathi Uppu(GU) in experimental rodents and to authenticate its safety behind its traditional use.

MATERIALS AND METHODS

Ghendhagam (Sulphur) and Kariuppu (Sodium chloride) were purified as per the procedure mentioned in the Siddha Literature. To purify Nellikai Ghendhagam, it was melted in the iron spoon with cow's butter and it was poured in a cow's milk. This procedure was repeated for 30 times. For each turn fresh cow's milk was used. To purify Kariuppu (Sodium chloride), it was dissolved in sea water, filtered and heated to attained the kuzhambu patham (Semisolid state) and dried in the sunlight. Purified Nellikai ghendhagm -1/2palam (17.5gm), purified kariuppu – 1 palam (35gm) was heated in the vessel. It was stirred well with rod until it mixed with each other. Then it was grinded in to a fine powder. [4]

TOXICITY STUDIES

The acute toxicity and chronic toxicity studies were carried out in pharmacological laboratory, department of Pharmacology, Government Siddha Medical College (GSMC), Palayamkottai. The experimental protocol was approved by the Institutional Ethical Committee (IEC), GSMC, Palayamkottai. (No.34/IEC/GSMC/2011-12 DT.27.06.2012).

EXPERIMENTAL ANIMALS

30Albino rats divided into 6 groups and 15 Albino rats divided into 3 groups weighing (80-120 gms) of either sex were used for acute and chronic toxicity study respectively. All the animals were maintained in a controlled environment condition of temperature (24±10C) an alternative 12h light/ dark cycles. They were reared at the animal house of department of Pharmacology, Government Siddha Medical College (GSMC), Palayamkottai. Animals were kept in cages and fed on commercial pellets (Hindustan Lever Ltd, Mumbai, India and water *ad libitum*.

PREPARATION OF STOCK SOLUTION

The *Siddha* drug *Ghendhiyathi uppu(GU) was* mixed uniformly in buttermilk which is an adjuvant for the test drug in such a way so as 2ml suspension contained 100 mg, 200mg,400mg,800mg, and 1600mg of test drug for acute toxicity study and whereas 2ml suspension contained dose of 100mg and 200mg for chronic toxicity study.

ACUTE TOXICITY STUDY

The acute oral toxicity study was carried out as per the OECD-425. The animals were segregated into six groups (Group I, Group II, Group III, Group IV, Group V, Group VI) consisting of five albino rats each. Group I is the control and Group II to Group VI were administered with a dose of 100mg, 200mg, 400mg, 800mg, 1600mg/100gm body weight of the animal respectively. Observations were made for physiological and behavioural responses and mortality for a period of 24 hours with the intervals of 1hr, 2hr, 4hr and 24hr. The LD50 was determined by observing the mortality rate in the drug treated groups.

CHRONIC TOXICITY STUDY

The rats were divided at random into a control group and two experimental groups with five animals in each group. The vehicle control group received 0.2% Carboxy methyl cellulose (CMC), whereas the experimental groups received *Ghendhiyathi uppu(GU)* (100mg, 200mg/100gm of body weight of the animal administered by means of bulged steel needle for 90 days. Body weight, haematological indices, behavioural changes were recorded on 0 day, 30^{th} day,60^{th} day, and at 90^{th} day after which the animals were fasted overnight after the dosage period. The animals were anesthetized with diethyl ether, and then decapitated. Paired blood samples were collected into heparinized and nonheparinized tubes. The heparinized blood was used for haematological

evaluation; the non-heparinized blood was allowed to coagulate, contents centrifuged, and the serum separated was analysed for biochemical parameters of the experiment.

DETERMINATION OF HEMATOLOGICAL PARAMETERS

The haematological parameters assayed included red blood cell (RBC) and white blood cell (WBC) counts inclusive of polymorph, nuclear leucocytes and lymphocytes, platelets, haematocrit and haemoglobin (Hb) estimation.

HISTOPATHOLOGY

One animal from each group was sacrificed and were necropsied at the end of the experiment after collection of blood samples and mentioned as 1A for 100mg, 1B for 200 mg, 1C for control groups. The viscera's like liver, heart, kidney was removed, weighed individually and fixed in 10% buffered formalin in labelled bottles. Organs were processed to study histological changes adopting paraffin method. The histopathological changes of these tissues were observed on gross and microscopic bases. The sections were stained with haematoxylin and eosin. The histopathology studies were carried out in the Department of pathology, Government Medical College, Tirunelveli.

RESULTS AND DISCUSSION

Table1 Acute toxicity study

No	Dose mg/100gm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	100	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
2	200	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
3	400	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
4	800	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
5	1600	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-

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

Chronic Toxicity study

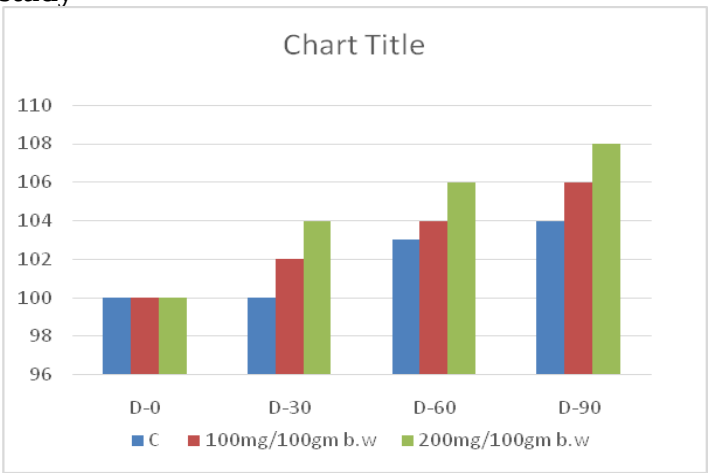


Fig-1: Changes in Body weight (gm) of *Ghendhiyathi uppu(GU)*treated Albino rats

Table 2. Effect of *Ghendhiyathi uppu(GU)* on Hematological parameters(control)

S.NO	Parameters	D-0	D-30	D-60	D-90
1	WBC total count	9800/cu.mm	9700/cu. Mm	9800/cu. mm	9800/cu. Mm
2	Differential Count				
	Neutrophil	25%	25%	23%	24%
	Eosinophil	-	-	-	-
	Basophil	-	-	-	-
	Lymphocyte	78%	78%	74%	74%
	Monocyte	-	-	-	-
3	Haemoglobin	74%	72%	72%	70%

Table 2.1 Effect of *Ghendhiyathi uppu* on Hematological parameters. (100mg/100gm b.w)

S.NO	Parameters	D-0	D-30	D-60	D-90
1	WBC total count	10200/cu.mm	10100/cu. Mm	10000/cu. mm	10000/cu. Mm
2	Differential Count				
	Neutrophil	28%	25%	25%	20%
	Eosinophil	-	-	-	-
	Basophil	-	-	-	-
	Lymphocyte	82%	84%	82%	80%
	Monocyte	-	-	-	-
3	Haemoglobin	74%	78%	76%	78%

Table 2.2 Effect of *Ghendhiyathi uppu* on Hematological parameters. (200mg/100gm b.w)

S.NO	Parameters	D-0	D-30	D-60	D-90
1	WBC total count	10200/cu.mm	10200/cu. Mm	10300/cu. mm	10200/cu. Mm
2	Differential Count				
	Neutrophil	26%	26%	22%	20%
	Eosinophil	2%	3%	3%	1%
	Basophil	-	-	-	-
	Lymphocyte	84%	80%	82%	79%
	Monocyte	-	-	-	-
3	Haemoglobin	76%	74%	74%	78%

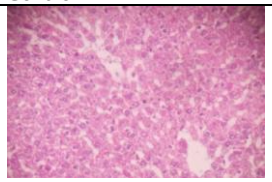
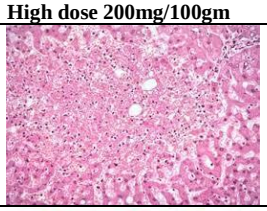
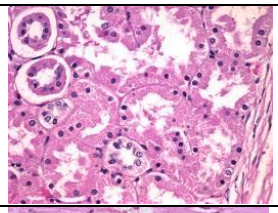
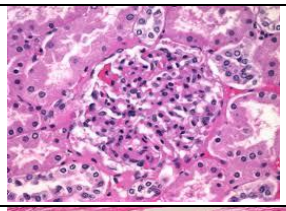
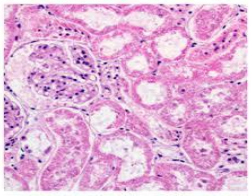
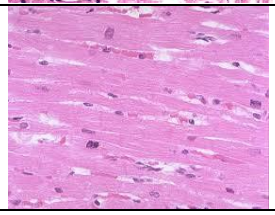
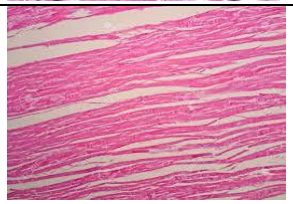
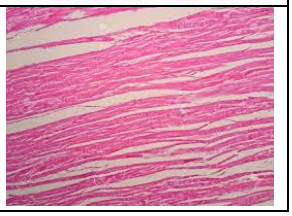
The Chronic toxicity study of *Ghendhiyathi uppu* with the above doses did not reveal any toxicity symptoms as revealed in body weights (Fig-1) and also organ weights of rats. Body weight and organ weight changes serve as an indicator of adverse side effects since animals that survive cannot lose more than 10% of the initial body weight. [5] No significant decrease in body weight has found among the control and treated group. Instead the body weights of experimental and control rats were found to be increased significantly indicating that there was no loss of appetite and suggesting that *Ghendhiyathi uppu(GU)* had no effect on the normal growth of rats, justifying the doses chosen.

The trial drug *Ghendhiyathi uppu(GU)* does not exhibit any significant toxicity as there was no abnormal behavioural changes or mortality after the oral administration of the drug up to a dose level of 1600 mg/100gm body weight throughout period of study (Table-1). Therefore, *Ghendhiyathi uppu* falls under class 4 of globally harmonized system (GHS) of classification and Labelling of chemicals.

Haematological and biochemical parameters play a decisive role in the drug induced toxicity Blood parameters analysis is highly relevant to risk evaluation in humans (91%) when assays involve rodents and nonrodents. [6] Damage to blood cells are detrimental to normal functioning of the body as blood forms the chief medium of transport for various drugs and xenobiotics in the body. [7] In the present study the Haematological (WBC, RBC, Hb) parameters, platelets, Lymphocytes, Monocytes, Eosinophils and basophils were found to show significant alteration at higher doses of 100mg and 200mg/100gm of body weight of the animal but the values were found to be well within the clinical range for rats[Table 2,2.1,2.2] This indicates that the test drug does not interfere with the production of blood cells (Haematopoiesis) and forecasts the associated safety when administered in the human body.

The histopathological analysis shows mild sinusoidal dilatation and congestion in liver, normal glomeruli with focal interstitial oedema in kidney and shows normal bundles of myocardial fibres in heart at the dose of 100mg/100gm body weight of the animal. At the dose of 200mg/100gm body weight of the animal, it reveals the presence of mild sinusoidal dilatation with focal necrosis of the liver, normal glomeruli with focal interstitial oedema with inflammatory cell infiltration in kidney and shows normal bundles of myocardial fibres.

Fig:2 Histopathological analysis

Organs	Control	Low dose 100mg/100gm	High dose 200mg/100gm
Liver			
Kidney			
Heart			

CONCLUSION

The present preclinical study aimed to validate the possible toxicity of the *Siddha* formulation *Ghendhiyathi uppu(GU)* in albino rat models showed that the drug did not show any signs of behavioural abnormality or mortality in 24 hours of Acute toxicity study with LD50> 1600mg/100gm body weight of the animal and also showed no significant alteration in the haematological, body weight and the behavioural abnormality in the chronic toxicity studies after repeated oral administration for 90 days. In chronic toxicity study shows mild histopathological studies in the tissues of the kidney and the liver at the dose of 200mg/100gm body weight of the animal. The dose administered for chronic toxicity studies in rats are relatively very high when compared to human dose level. The aim of giving such a high dose was to find out the type of toxicity produced by it. All these changes are warning about the adverse effect of *Ghendhiyathi uppu(GU)* in long term administration at clinical side. Hence the tested *Siddha* formulation *Ghendhiyathi uppu(GU)* considered as relatively safe for human consumption as the prescribed dose mentioned in Siddha literature and above this dose level is not safe in long term use.

ACKNOWLEDGEMENT

The author thankfully acknowledges Dr. M. Thiruthani MD (Siddha), Vice-principal, Government Siddha Medical College, Palayamkottai for his continuous guidance throughout this study.

REFERENCES

1. Narayansami V. In: Pandit SS, editor. Introduction to the Siddha System of Medicine. T. Nagar, Madras: Anandam Research Institute of Siddha Medicine, 1975; 1-51.
2. Thas, J.J., Siddha medicine—background and principles and the application for skin diseases. Clinics in dermatology, 2008; 26(1): 62-78.
3. Deva asirvaatham samuvel, Marunthu sei iyalum noolum, published by Directorate of Indian Medicine and Homeopathy, Chennai,2016;14-24
4. Thiyagarajan R, Gunapadam thaathu- jeeva vaguppu Part 2,3, Directorate of Indian Medicine and Homeopathy, 4th edition, 2004; 305,387
5. Wonder H.K. M. George K.A, and Gyasi E.B., Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of Hilleria latifolia (Lam.) H. Walt. (Phytolaccaceae) in rodents West African Journal of Pharmac, 2011; 22(1): 27–35.
6. Olson. H, Betton. G, D. Robinson et al., Concordance of the toxicity of pharmaceuticals in humans and in animals.

Regulatory Toxicology and Pharmacology, 2000; 32(1): 56–6.
7. Adeneye A. A., Ajagbonna O. P, Adeleke T. I., and Bello S. O. Preliminary toxicity and phytochemical studies of the stembark aqueous extract of Musanga cecropioides in rats, Journal of Ethnopharmacology, 2006; 105(3): 374–379.

How to cite this article: Thilagavathi. R, Kavitha. T, Esaivani. S et. al. Toxicological profiling of a *siddha* formulation *ghendhiyathi uppu* in experimental rodents. International Journal of Research and Review. 2020; 7(3): 145-150.

******