

Research article

Preclinical toxicological screening of Siddha medicine *Ganthi Mezhu* by acute toxicity studies in Wistar albino ratsS. Yavanarani¹, R. Selvakumar², M. Sathiyabama³¹Yavanarani Siddha Clinic, Pillaiyarpalayam, Kanchipuram, 631501, Tamil Nadu, India²Siddha Government Hospital, Tiruttani, Tiruvallur, 631209, Tamil Nadu, India³Siddha Physician, Panruti, Cuddalore, 607106, Tamil Nadu, India

(Received: April 2022

Revised: January 2023

Accepted: February 2023)

Corresponding author: R. Selvakumar. Email: dr.selvakumar7@gmail.com

ABSTRACT

Introduction and Aim: Siddha formulations of drugs had been used for hundreds of years in international conventional drugs for his or her capacity fitness benefits. *Ganthi Mezhu* (GM) has a wide range of therapeutic properties. The objective of the present research was to establish the safety profile of the experimental drug GM in a rat model by acute and sub-acute oral toxicity, as per OECD regulatory guidelines.

Materials and Methods: Acute toxicity testing is done for an animal species to determine the effects of a single dose on that species. In this investigation, acute toxicity was assessed using single oral administrations of 50 mg/kg, 300 mg/kg, and 2000 mg/kg. In the subacute investigation, the test substance GM was repeatedly dosed (4.68, 23.4, and 46.8 mg/kg/day) for 28 days followed by these hematological and biochemical parameters were assessed.

Results: The study's findings showed that after administering the test substance GM to experimental animals once or repeatedly, there was no evidence of toxicity and no deaths. The subacute toxicity study's GM treated groups' normal hematological and serological profiles provided additional evidence that the formulation is not harmful.

Conclusion: The finding of the study supported the notion that Siddha formulation GM is non-toxic and has a broad spectrum of safety.

Keywords: Toxicity assessment; acute; sub-acute; *Ganthi Mezhu*; hematological; biochemical.

INTRODUCTION

Siddha medicine is one of the ancient traditional therapeutic systems extensively employed in Southern India and parts of Sri Lanka, Malaysia, and Singapore. This system is an exceptional Indian system of medicine that is highly effective and provides physical, mental and mental comfort. This system was established by 'Siddhar', known for its diverse capabilities. They are masters of natural chemistry and have used a variety of natural resources such as plants, animals and minerals to formulate medicinal products (1). Gandagam (sulfur) is a crystalline non-metal used in Siddha formulations such as Lasayanam (semi-dried powder), Machirai (pill), Mejug (wax-like drug), Palpam (baked powder), and Cendulum (red powder). It possesses bitter and astringent taste Gandagam (sulfur) must be purified by the various purification methods available in the Siddha system before it can be used in medicinal formulations. It is an invariable component of the majority of Siddha formulations (2).

There are 64 types of formulations, 32 of which are internal medicine and 32 are external medicines. Of these formulations, 'Mezhu' is an internal medicine that is said to have a waxy viscosity with a shelf life of 5 years. It can be made by various treatments i.e., mechanical, phyto, photo, heat, acid,

salt, etc., (3). *Ganthi Mezhu* has a wide range of therapeutic properties such as anti-aging; improves digestion, treatment of illnesses such as chronic fever, urinary tract infection, skin disorders, diarrhea, itching, hemorrhagic disorders, eczema, leprosy, cystic acne and oligospermia. It also acts as a very good blood purifier and treats scabies.

Toxicology is a field of science that helps to understand the adverse effects of chemicals or substances or agents on people, animals, and other living organisms. The fundamental element of this process is the characterization of the relationship between exposure (or dose) and response. Regulatory requirements clearly endorse the need to profile the toxicity of a drug for clinical efficacy before it is intended for use in humans (4). Therefore, it is crucial that the medication demonstrates both safety and effectiveness before being prescribed for use in therapeutic settings. This study's primary goal was to assess the *Siddha Gandhaga Mezhu* formulation's short- and long-term safety using acute and chronic toxicity tests in Wistar albino rats.

MATERIALS AND METHODS**Drug**

The raw drug of *Ganthi Mezhu* obtained from Siddha medical shop, Chennai, Tamil Nadu, India. A Department of Medicinal Botany of the National

Institute of Siddha identifies and authenticates these raw drugs *Ganthi Mezhu*g, and then the medicine was purified and prepared in Gunapadam Laboratory of the same Institution. The trial drug *Ganthi Mezhu*g was stored in clean and dry glass bottles.

Acute toxicity

Albino Wistar rodents weighing between 150 and 200 g were used in this research. The plastic enclosures in which the animals were acclimated were completely ventilated with fresh air. In addition, the animals were maintained at room temperature with a relative humidity of 50% to 70% and on a 12-hour dark/light schedule. All macro and micro nutrients were given by pellet feed in a normal composition for the seven days preceding the start of the research, along with water at will. The tests were conducted after receiving the necessary approval from the NIS IAEC (1248/AC/09/CPCSEA-9/Dec/2013/23).

A total of 24 albino Wistar rodents were randomly assigned to one control group, three therapy groups, and three control groups, each with three male and three female members. The rodents were allowed unrestricted access to water while fasting for 12 to 16 hours. Rats in groups II, III, and IV received oral doses of the research medication *Ganthi Mezhu*g (GM) at 50 mg/kg, 300 mg/kg, and 2000 mg/kg (p.o.), respectively. Group I served as the reference group. For 72 to 14 days, the animals were constantly observed for any indications of behavioral changes, weight changes, or mortality.

Repeated oral toxicity study

A 28-day repeated oral toxicity analysis was carried out using OECD standards - 407 with a few small changes (5). For the therapy of skin disorders, people were advised to take 520 mg/day of GM, and rats were given doses of 4.68 mg/kg that were calculated using the total surface area of the person (6). In this research, albino Wistar rats (150-200g) were split into four groups of ten animals, with five males and five females in each group, and received three dosages of GM, namely 4.68, 23.4, and 46.8 mg/kg/day for both sexes. In Group I, saline was given orally, while in Group II, III and IV, GM was

given orally at 4.68, 23.4 and 46.8 mg/kg/day, respectively, for 28 days. As part of the experimental protocol, animal mortality and morbidity were monitored twice daily. At the conclusion of the predetermined treatment period, sedated animals that had been fasting the previous night (with access to water) were used to gather blood samples via a retro-orbital puncture into heparinized tubes.

Blood parameters

In order to measure blood parameters, a fully automated hematology analyzer was used to measure total HB, total RBC, total WBC, platelets (PLT), PCV, MCV, MCH and MCHC.

Biochemical Investigation

An estimation of biochemical parameters was carried out using serum. A standard protocol was followed to measure cholesterol and glucose levels, BUN, creatinine, bilirubin levels in serum samples of control and experimental rats. A Colorimetric method was performed to assess the SGOT, SGPT and ALP levels in serum samples of control and experimental rats.

Statistical analysis

Statistical analysis is performed on hematological and metabolic variables. Findings are presented as mean and SD, with a 0.05 p-value when compared to control rodents (n = 10).

RESULTS

There was no mortality observed at 50 mg/kg, 300 mg/kg, or 2000 mg/kg in the acute toxicity study. Furthermore, no significant change with regard to clinical signs of acute toxicity was observed in a short period of time (24-48 h) and a long period of time (14 days).

Hematological parameters

For assessing blood parameters, *Ganthi Mezhu*g was administered to the rats at four doses of 4.68, 23.4, and 46.8 mg/kg/day without exhibiting any statistically significant difference in these parameters (Table 1).

Table 1: Hematological parameters of Wistar albino rats group exposed to *Ganthi Mezhu*g

Parameters	Control	4.68mg/kg	23.4 mg/kg	46.8 mg/kg	P-value
Hemoglobin(g/dl)	12.3±1.67	11.83±1.67	12.61±0.40	12.8±0.77	NS
Total WBC (cells/cu.mm)	7.45±1.56	7.65±1.825	7.625±1.295	7.43±1.21	NS
Neutrophils (%)	65.3±6.77	64.30±6.33	67.85±11.57	61.483±5.31	NS
lymphocytes (%)	25.3±5.66	27.53±5.66	25.5±7.59	30.43±4.89	NS
Monocytes (%)	1.8±0.56	1.8±0.86	1.68±0.83	2.216±0.381	NS
Eosinophils (%)	2.1±1.23	4.1±2.44	2.5±1.23	3.85±4.977	NS
Basophils (%)	0.23±0.04	0.11±0.04	0.116±0.04	0.2±0.08	NS
Platelets cells/ul	335.13±60.23	335.16±114.48	345±40.18	337±67.87	NS

Total RBC (cells/cu.mm)	4.56±0.45	4.535±0.49	4.645±0.322	4.87±0.56	NS
PCV%	43.35±7.46	39.2±6.56	41.91±3.30	43.63±7.74	NS
MCV	89.4±16.0	89.75±14.68	98.91±5.82	98.86±6.44	NS
MCH pg	25±1.36	24.96±4.48	28.4±0.76	27.51±1.15	NS
MCHC g/dl	27.2±2.90	27.6±2.04	28.51±1.23	28.56±1.105	NS

NS - No Significant ($p > 0.05$), $n = 10$ values are mean $\pm$ S.D

Biochemical parameters

In rats treated with GM at 4.68, 23.4, and 46.8 mg/kg/day, there were no statistically significant differences in biochemical parameters (Table 2).

Table 2: Biochemical parameters of Wistar albino rats group exposed to *Ganthi Mezhuugu*

Biochemical parameters (mg/dl)	Control	4.68mg/kg	23.4 mg/kg	46.8 mg/kg	P-Value
Glucose	80±2.34	78±2.966	96.33±3.93	101.96±2.99	NS
Cholesterol	130±33.23	123±0.045	130±0.045	140±0.06	NS

NS- No Significant ($p > 0.05$), $n = 10$ values are mean $\pm$ S.D

Serum renal biochemistry profile of rats in sub-acute oral toxicity study

Rats treated with GM at 4.68, 23.4 and 46.8 mg/kg/day showed no statistically significant differences in serum renal biochemistry parameters of creatinine and BUN levels (Fig. 1 and 2).

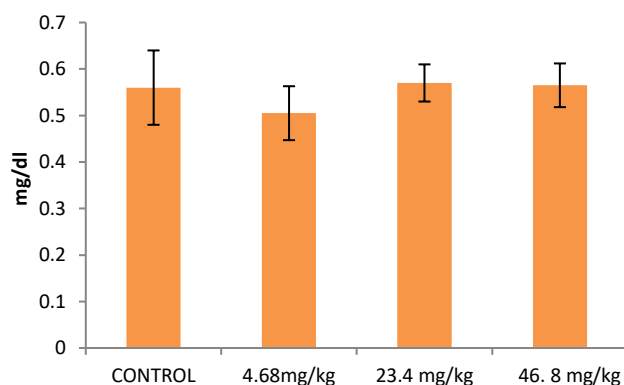


Fig. 1: Creatinine

Serum hepatic biochemistry profile of rats in sub-acute oral toxicity study

In rats treated with GM at 4.68, 23.4, and 46.8 mg/kg/day, there were no statistically significant differences in serum hepatic biochemistry parameters of the levels of bilirubin, SGOT, SGPT and ALP (Fig 3, 4, 5 and 6).

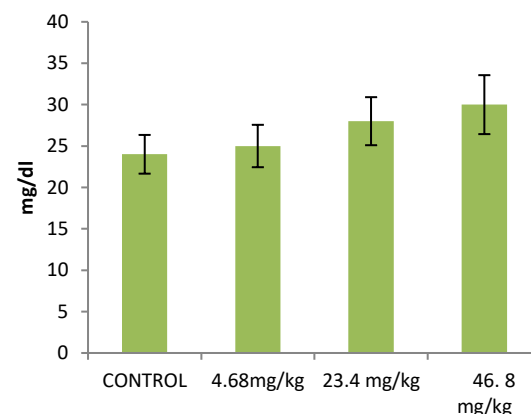


Fig. 2: BUN

Values represented mean $\pm$ SD of three independent experiments with ten replicates each.

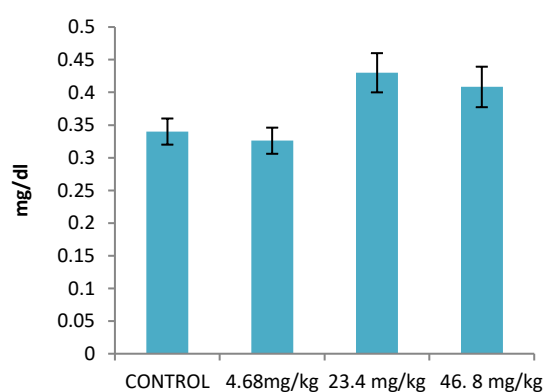


Fig. 3: Bilirubin

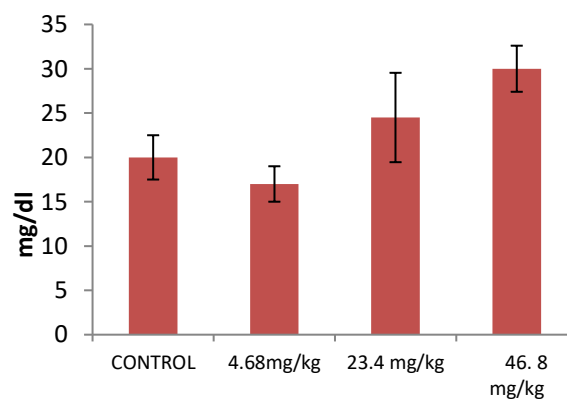


Fig. 4: SGOT

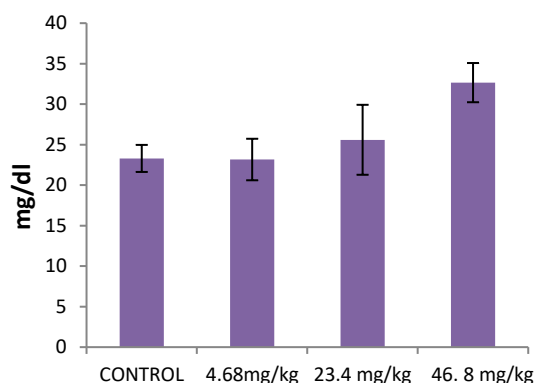


Fig. 5: SGPT

Values represented mean $\pm$ SD of three independent experiments with ten replicates each

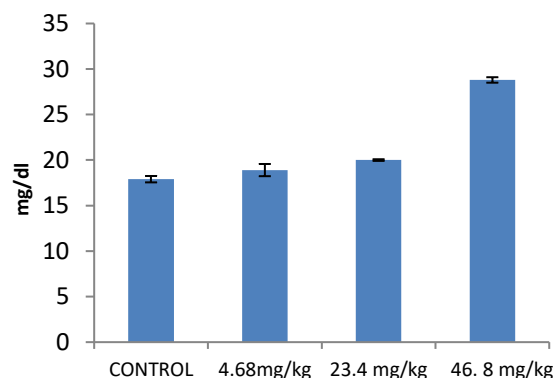


Fig. 6: ALP

DISCUSSION

Every drug that deals with human life must prove safe, even WHO strongly emphasizes safety rather than efficacy. Each year, approximately 10,000 molecules are identified, but only one is approved for use after completing preclinical and clinical trials. It was found at the preclinical and clinical levels that the drug had adverse toxic effects, which made the remaining 99% fail. Because siddha preparations have been prescribed to a large group of people for several years, the investigator needed to justify their safety for humans and animals in the toxicity study in rodents, in order to establish a safety margin. Toxicology studies are frequently carried out on Siddha formulations to ascertain whether the preparation's inherent chemical components could have negative impacts on the body (7). The first stages in assessing the toxicology of herbal remedies have been characterized as acute toxicity and LD50 calculations, and the outcomes of such studies offer thorough information on how these products are categorized based on their toxicological characteristics (8).

It was observed that after the 14-day post-dose period, no mortality occurred in rats treated with *Ganthi Mezhu*g, which was administered at the doses of 50 mg/kg, 300 mg/kg, and 2000 mg/kg in accordance with OECD guideline 423. Aside from that, none of the measured parameters including body weight, food and drink consumption, social, sensory, and motor behavior, muscle strength, exploratory behavior, etc., were substantially impacted. The typical lifespan of experimental animals may be influenced by visible side effects that may come from acute studies that reveal dosing protocols, target organ toxicity, and target organ toxicity. Hence, this study evaluated the toxicity of Siddha GM in rats at doses of 4.68, 23.4, and 46.8 mg/kg/day for 28 days. Body weight changes are vital indicators of toxicity and can also provide information about animal welfare (9,10). According to the non-significant difference in the mean body weight gain in all treatment groups, the GM test drug did not affect the animals' normal metabolism. It is possible that their

weight was attributed to nutritional components in their diet and the palatability of the GM test drug, which were closely related to the non-significant difference in this parameter.

Studies of blood parameters could be able to determine the unfavorable impact of foreign chemicals in plant extracts on an animal's blood components (11,12). Blood toxicity is indicated by the RBC and Hb counts. They have an impact on the shape, osmotic instability, and incorporation of hemoglobin into red blood cells. The oxygen-carrying capability of the blood is indicated by the RBC count, and the oxygen delivery to organs following administration of the intact GM test drug is indicated by the Hb count.

In this research, there were no notable changes in any of the hematological parameters, including RBC, WBC, and platelet count, Hb, PCV, MCV, MCH, and MCHC, following therapy with GM at three dosage levels of 4.68, 23.4, and 46.8 mg/kg/day.

Similar phenomenon has also been reported during acute toxicity evaluation of other siddha formulations viz., *Purna Cantirota Centuram* (13), *Maruthuva Sudar Chooranam* (14) and *Maampisin Chooranam* (15).

The analysis of hepatic and renal function is the chief source of the toxicity analysis of any herbal preparation as they help to keep normal physiology functioning properly (16). In a sub-acute toxicity study, treatment with GM at 4.68, 23.4, and 46.8 mg/kg/day did not cause any significant changes in the lipid profile, cholesterol levels, renal function or liver enzyme levels in both male and female rats. The results obtained were agreement with the previous reports by other investigators i.e., Gayathiri *et al.*, in *Sirupeellai Samoola Kudineer* (17), Tharakan *et al.*, in *RasaGanthi Mezhu*g (18) and Vijibala *et al.*, in *Sambirani Poo Kuligai* (1).

CONCLUSION

Overall, the current research clearly shows that Siddha product *Ganthi Mezhu*g is well tolerated in acute toxicity studies at the doses of 50 mg/kg, 300

mg/kg, and 2000 mg/kg in Wistar albino rats. It can be found that the test drug GM does not cause any treatment-related adverse effects based on hematological and biochemical observations at the doses examined after its repeated daily oral administration for 28 days in the animals. As a result, it may be categorized as being relatively safe and practically nontoxic for consumption.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Director and faculty, Govt. Siddha Medical College, Chennai for their extensive support and advice throughout this study. We also acknowledge our sincere thanks to Dr. N. Yogananth, Mohamed Sathak College of Arts and Science, Chennai, Tamil Nadu, India for his technical assistance in publishing this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

- Vijibala, M., Karolin Daisy Rani, R., Velpandian, V., Pitchiah Kumar, M. Preclinical toxicological profiling of Siddha formulation Sambirani Poo Kuligai by Acute and Sub-acute toxicity studies in accordance with OECD Guidelines. *Int J Curr Res Med Sci* 2018; 4(12): 1-10.
- Rajalakshmi, P., Abeetha, M., Devanathan, R. Physicochemical analysis of gandhagam before and after purification. *Int J Curr Pharm Res* 2010; 2(4): 32-35.
- Shanmugapriya, P., Christian, G.J., Vajrai, R., Brindha, P., Elansekar, S., Murugesan, M. *et al.*, Antimicrobial efficacy of Gandhagam (raw sulphur), purified Gandhagam and Gandhaga Mezhugu - a traditional Siddha formulation. *J Pure Appl Microbiol* 2013; 7: 1-4.
- Saganuwan, S.A. Toxicity studies of drugs and chemicals in animals: an overview. *Bulgarian Journal of Veterinary Medicine* 2017; 20: 291-318.
- Organization for Economic Cooperation and Development; Guidelines for the Testing of Chemicals/Draft Updated Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. 2008.
- Freireich, E.J., Gehan, E.A., Rall, D.P., Schmidt, L.H., Skipper, H.E. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother Rep.* 1966; 50(4): 219-244.
- Iniaghe, O.M., Egharevba, O., Oyewo, E.B. Effect of aqueous leaf extract of *Acalypha wilkesiana* on hematological parameters in male Wistar albino rats. *British Journal of Pharmaceutical Research* 2013; 3(3): 465-471.
- Ukwuani, A.N., Abubakar, M.G., Hassan, S.W., Agaie, B.M. Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*. *International Journal of Pharmaceutical Science and Drug Research.* 2012; 4(4): 245-249.
- Seewaboon, S., Nirush, L., Umarat, S., Amornat, T., Anongnad, N., Nadthanganya, S. Acute and subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diets in rats. *Songklanakarin Journal of Science and Technology* 2008; 30(5): 729-737.
- Sharaibi, O.J., Ogundipe O.T., Magbagbeola, O.A., Kazeem, M.I., Afolayan, A.J. Acute and subacute toxicity profile of aqueous leaf extract of *Nymphaea lotus* linn (Nymphaeaceae) in Wistar rats. *Tropical Journal of Pharmaceutical Research* 2015; 14(7): 1231-1238.
- Ashafa, A.O.T., Yakubu, M.T., Grierson, D.S., Afolayan, A.J. Effects of aqueous extract from the leaves of *Chrysocoma ciliata* L. on some biochemical parameters of Wistar rats. *African Journal of Biotechnology* 2009; 8(8): 1425-1430.
- Saheed, S., Oladipipo, A.E., Abdulazeez, A.A., Olarewaju, S.A., Ismaila, N.O., Emmanuel, I.A., *et al.*, Toxicological evaluations of *Stigma maydis* (corn silk) aqueous extract on hematological and lipid parameters in Wistar rats. *Toxicology Reports* 2015; 2: 638-644.
- Chitra, B., Ramaswamy, R.S., Suba, V. Toxicity Evaluation of Purṇa Cantīrotaya Centuram, a Siddha Medicine in Wistar Rats. *International Scholarly Research Notices* 2015; 473296.
- Dhivyalakshmi, K., Arul Jothi, S., Anbu, N., Sivaraman, D. Preclinical safety evaluation of Sarabendra Siddha Maruthuva Sudar Chooranam by acute and subacute repeated oral toxicity studies in rodents. *Int J Curr Res Bio. Med* 2019; 4(10): 1-12.
- Dhivyabharathi, A., Brunda, S., Anbu, N., Kanakavalli, K. Preclinical toxicological screening of Siddha Formulation Maampisin Chooranam by acute and sub-acute toxicity studies in Wistar rats. *Int J Adv Res Biol Sci* 2018; 5(9): 111-120.
- Oyagbemi, A.A., Omobowale, T.O., Azeez, I.O., Abiola, J.O., Adedokun, R.A., Nottidge, H.O. Toxicological evaluations of methanolic extract of *Moringa oleifera* leaves in liver and kidney of male Wistar rats. *J Basic Clin Physiol Pharmacol* 2013; 24: 307-312.
- Gayathri, V., Muthulakshmi, V., Chandronitha, C., Vasanthkumar, M., Ramakrishnan, G., Ananthi, S., *et al.*, Toxicological evaluation of an antilithiatic polyherbal Siddha formulation-Sirupeellai Samoola Kudineer in experimental rats. *Human and Experimental Toxicology* 2011; 30(8), 952-964.
- Tharakan, S.T., Kuttan, G., Kuttan, R., Kesavan, M., Austin, S.R., Rajagopalan, K. Toxicity Studies of Siddha Medicine - RasaGanthi Mezhugu. *The Open Toxicology Journal* 2010; 4: 43-50.