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RESEARCH ARTICLE

Acute and Sub-acute Toxicity Studies of a *Siddha* Medicine *Ganthaga Mezhugu* by Oral Administration in Sprague Dawley Rats

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Abstract

Psoriasis, a chronic auto-immune skin disease, is troubling 1-3 % of the world population with a 0.44-2.8% prevalence rate in India affecting more males than females. It is considered a dermatologist's menace as it is challenging to treat the condition. *Siddha*, one of the traditional systems of medicine practised more in South India, includes a wide range of medicines that are used to treat or manage various ailments, including psoriasis. One such medicine is *Ganthaga Mezhugu (GM)* used in treating skin diseases like psoriasis. This present study deals with the evaluation of the safety of *GM* as per the Organization for Economic Co-operation and Development (OECD) Guidelines 423 and 407 in Sprague Dawley (SD) rats. In the Acute Toxicity study, SD female rats were grouped into five, in which Groups I and II received elemental Sulphur in two different doses, Groups III and IV received Sulphur 'purified' with two different *Siddha* processes and Group V received *GM* (2000mg/kg b.wt.). In the sub-acute toxicity study, SD rats of both sexes were grouped into six in which Group 1 served as vehicle control, Groups 2, 3, and 4 as low, mid, and high dose groups and the last two (Groups 5 and 6) as satellite control and satellite high-dose groups. In the acute study, no mortality, toxic signs or any gross pathological changes were noted. Hence, the LD_{50} value of *GM* was found to be greater than 2000mg/kg b.wt. In the sub-acute toxicity study, no mortality or morbidity occurred. There was a gradual increase in body weight with normal food and water intake indicating normalcy in its metabolism. There were no significant changes in hematological and biochemical parameters, serum electrolytes and gross pathology. Also, no pathological changes were found in the histopathology of organs in treated animals when compared with control group animals. Based on the results, regarding the Globally Harmonized System of Classification and Labelling of chemicals, *GM* can be classified as Category−5, which implies its safety for human consumption. Moreover, the results of the sub-acute study also confirm the safety of *GM* up to the dose of 400mg/kg b.wt.

Keywords: OECD, Sulphur, Toxicity, Traditional Medicine

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1. Introduction

Siddha is a unique Indian system of medicine which is very potent and provides a holistic treatment that comforts the body, mind, and soul. This system was founded by '*Siddhars*', who are known for their multitalented abilities. They are masters in natural chemistry and formulated medicinal preparations utilizing various natural resources of plants, animals, and minerals. So, these wide ranges of medicines in *Siddha* are preferred for various ailments, including skin diseases, like psoriasis. Psoriasis is a chronic auto-immune skin disease, which is considered a dermatologists' menace as it poses a tough challenge to manage. In *Siddha*, this condition is considered congruent to *Kalanjagapadai*[1]. Clinical symptoms of *kalanjagapadai* draw parallels with the features of psoriasis. It is an auto-immune disorder characterized by thickened red plaques with overlying silver-white scales. Commonly 90% of the cases of psoriasis are categorized under psoriasis vulgaris[2], which affects both sexes equally and people of all ages; it causes considerable impacts on the quality of life.

Psoriasis is affecting 1-3 % of the population worldwide and with a prevalence of 0.44-2.8 % in India. It commonly affects individuals in their third or fourth decade with males being affected two times more commonly than females. Psoriasis significantly impairs the patient's and their family member's quality of life resulting in great physical, emotional, and social burdens[3]. To overcome this, *Siddha* medicines are being used clinically since ancient times. One such medicine being used in chronic skin diseases is *Gandhaga Mezhugu* (*GM*)[4]. According to *Siddha* literature, *GM* is indicated for *Thol Noikal* (skin diseases) more than *Gandhagam* (sulphur) alone is indicated for 18 types of *Kuttam* (skin disease)[5]. Being indicated for skin diseases, the drug *GM* must be administered for a minimum of 48 days (one *mandalam*) as per *Siddha* treatment modalities. However, there is no scientific report that showed the evaluation of safety or human toxicity. Even though we were using this medicine clinically for many decades, we need to validate its safety through preclinical safety studies as it is mandatory as per the traditional drug development process. This traditional methodology is very much similar to "Reverse Pharmacology", which emphasizes bedside to laboratory approach. Hence, the present study was undertaken to establish the safety profile of *GM* in experimental animals, which will contribute strong evidence for its safety in clinical use.

2. Materials and Methods

2.1 Authentication of Raw Drugs

Sulphur was identified and authenticated by a geo chemist at SASTRA University. Then, herbs used in purification (ginger, white onion) and processing (banana rhizome) of Sulphur were authenticated at the Plant Anatomy Research Centre (PARC), Tambaram by Prof. Dr. P. Jeyaraman, botanist by both observing the macroscopic and microscopic features of randomly selected samples.

2.2 Preparation of Medicine

After purifying Sulphur using butter and banana rhizome juice as per the literature[5], it was ground in an electric motor by adding white onion juice little by little for 120 hrs and subsequently with ginger juice for 120 hrs. After the rubbing was over till it attained a waxy consistency and retained the same. It was preserved in an airtight bottle.

2.3 Experimental Animals Husbandry

Young adult male and female (non-pregnant and nulliparous) Sprague Dawley (SD) rats of 140-160 g body weight, obtained from *in vivo* Biosciences (CPCSEA, Reg. No: 1165/PO/RCBI/S/08/CPCSEA), Bangalore, India, were used in this study. Animals were housed individually in polypropylene cages in a well-ventilated room with artificial photoperiod of 12h light/12h dark cycle under an ambient temperature (23±2 ˚C) and humidity (50-60 %). 12-15 air changes/hr were maintained in animal confinements. De-dusted and autoclaved paddy husk was used as bedding material. Rats were fed with a standard rodent pellet diet (Nutrilab Rodent, Tetragon Chemie, India) and were given purified water *ad libitum*. The animals were acclimatized in the experimental room for a period of 7 days before the beginning of the study. Guidelines of "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were stringently followed throughout the study. Institutional Animal Ethical Committee, Centre for Toxicology and Developmental Research (CEFT), Sri Ramachandra University, Chennai, India, has approved the study (IAEC/50/SRU/531/2017).

2.4 Acute Oral Toxicity Study

Acute oral toxicity was performed according to Organization for Economic Co-operation and Development (OECD) guideline - 423 for testing of chemicals[6]. Six young female adult rats weighing 140-160 g were randomized into control and test groups (3 animals/group) before the test drug administration. The control group received 0.5% carboxymethyl cellulose (0.5% CMC) as a vehicle. The test group was categorized into five groups based on the test drug administered. Groups I and II were administered with raw Sulphur of dose 2000 mg/kg b.wt. and 5000 mg/kg b.wt. respectively. Group III and IV were administered with purified Sulphur which differs in the herbs used for purification namely Henna curd and banana rhizome juice respectively. Group V was administered with the finished product *GM*. All the test drugs were administered via gastric intubation. All the test group and control group animals were observed for mortality and clinical signs of toxicity such as convulsion, tremor, straub tail, sedation, excitation, abnormal gait (rolling and tiptoe), jumps, motor coordination, loss of balance, fore paw treading, writhes, piloerection, stereotypies (chewing and head movements), head twitches, scratching, respiration, aggressiveness, fear, reactivity to touch, muscle tone, loss of righting reflex, ptosis, exophthalmos, loss of grasping, akinesia, catalepsy, loss of traction, loss of corneal reflex, analgesia, defecation, salivation, lacrimation, and others. All the animals were observed for these features at 30 min, 1, 2 and 4 hrs, and subsequently one time a day for the upcoming 14 days following vehicle or test drug administration. The changes in body weight were monitored and recorded on Days 0, 1, 2, 7 and 14. At the end of 14 days, the experimental animals were euthanized and gross pathological changes were investigated in the vital organs such as the brain, eyes, salivary gland, lymph node, trachea, oesophagus, lungs, heart, liver, stomach, pancreas, duodenum, small and large intestine, kidney, adrenal, spleen and sex organs. LD50 cut-off value of all five test drugs were determined in accordance with Globally Harmonised System of Classification and Labelling of chemicals[7].

2.5 Repeated Dose 28 Day Oral Toxicity Study

The repeated dose 28 day oral toxicity study was conducted according to OECD test guideline 407[8]. In *Siddha* literatures, 500mg of *GM* was advised to administer for the treatment of psoriasis in adult humans. In the present study, three dose levels (100, 200 and 400 mg/kg body weight/day) of *GM* were arrived and fixed as low, mid, and high doses respectively, as per the OECD guideline. Sprague Dawley rats of both sexes were divided into six groups with 10 animals (5 male + 5 female) in each group. Since *GM* was soluble and stable in water, it was used as a vehicle. The solubility and homogeneity of *GM* in vehicle was ensured while dosing. Group I served as control group and received water as vehicle. Groups II, III, and IV were administered with 100, 200, and 400 mg/kg/b.wt. of *GM* respectively, via gastric intubation for 28 successive days. In this present study, Group V was considered as satellite control receiving water as vehicle for *GM* and Group VI was known as High dose Satellite group receiving *GM* at 400 mg/kg.b.wt./day, P.O for a period of 28 days. Later, these Satellite group animals (Control and high dose) were observed for additional 14 days without dosing to identify reversibility or persistence of any toxic effects.

Animals in all the groups were observed for mortality and morbidity twice a day till the day of necropsy. They were also observed for the clinical signs of both Home cage observations (Circling, convulsion, tremor, ataxia, walking backward, hunched posture, kyphosis, lordosis, vocalization, and piloerection) and Hand held observations (self-mutilation, skin-erythema, skin swelling,exophthalmos, lacrimation, chromodacryorrhea, visible mucous membrane for paleness, cyanosis and icterus, dyspnea, nasal discharge, salivation, bleeding from external orifices, and diarrhoea), before and after *GM* exposure on day 0 and daily once till the day of necropsy.

Body weight of the animals was recorded on day 0, day 1, and thereafter weekly once till the day of necropsy. Food and water consumption were observed daily. At the end of 28 days, the animals of Groups I-IV were fasted overnight with free access to water. Then they were all subjected to blood sample collection through retro orbital puncture in heparinised (for haematological and biochemical analysis) and non-heparinised tubes (for serum electrolytes). Groups V and VI were kept for overnight fasting (water allowed) on 42nd day prior to blood collection. The hematological parameters such as Hematocrit (HCT), Hemoglobin (HGB), total erythrocyte count (RBC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), platelet count

(PLT), reticulocytes, total leucocyte count (WBC), differential (neutrophils, lymphocytes, eosinophils, basophils and monocytes) leucocyte count and blood clotting time were measured for all the animals. The biochemical parameters such as glucose, total cholesterol, triglycerides (TGL), total protein, albumin, glutamyl pyruvate aminotransferase/alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GT), total bilirubin, creatinine, urea were measured in serum of test animals. Serum electrolytes such as total calcium, potassium, sodium, chloride and inorganic calcium were analyzed.

2.5.1 Histopathology

Necropsy was done on the 29th day for all animals except the satellite group (Control and High dose), which was done on the 43rd day. After collecting blood, all the animals were euthanized and subjected to detailed gross necropsy, which includes a gross examination of external orifices, the cranial, thoracic and abdominal cavities, and their contents. Absolute wet weight of the organs such as the brain, heart, liver, paired kidneys, paired adrenals, spleen, paired testes, paired epididymis, male sex glands (prostate + seminal vesicles with coagulating glands as a whole), uterus with the cervix, paired ovaries, and thymus were recorded from which respective relative organ weight was calculated. Full histopathology was carried out on the preserved organs and tissues of all animals in the control and high-dose groups initially. If any treatment-related changes were observed in the high-dose group, then the low-dose and mid-dose group were to be studied for histopathological changes. The organs were preserved in 10% neutral buffered formalin, trimmed and sectioned into a 5µ thickness of tissue which was stained with hematoxylin and eosin for histopathological investigation.

2.5.2 Statistical Analysis

Data were expressed in mean ± Standard Error Mean (SEM). The Unpaired Student 'T' test was used to compare the mean difference between the vehicle and *GM*-treated groups. $p<0.05$ and $p<0.01$ were considered to be significant. All statistical analyses were performed with the commercially available software Graph Pad Prism 4.0.

3. Results

3.1 Acute Oral Toxicity Study

There were no toxicity signs or mortality was reported in the acute oral toxicity study with raw sulphur even at the dose of 5000mg/kg.b.wt. Similarly, no toxicity was developed in animals treated with sulphur purified with Henna-curd, sulphur purified with banana rhizome juice and *GM* at 2000mg/kg.b.wt. The animals gained body weight gradually but with no significant difference (Figure 1). In addition to that, no abnormal changes were found in gross pathology. Hence, the LD50 value of *GM* was found to be greater than 2000mg/kg b.wt. With reference to the Globally Harmonised System of Classification and Labelling of chemicals, *GM* can be classified as Category–5 and hence proves its safe consumption in humans.

3.2 Repeated Dose 28 Day Oral Toxicity Study

All the animals were appeared normal and didn't exhibit any signs of toxicity during the observation period of 28 days. This implicates that there was no test drug related morbidity or mortality observed in any of the animals of both sexes treated with 100, 200 and 400 mg/kg b.wt. orally for uninterrupted 28 days. The same was experiential in the satellite group also. No significant change in the food and water consumption when compared to the control group of animals. There was a gradual increase in the body weight of all the group

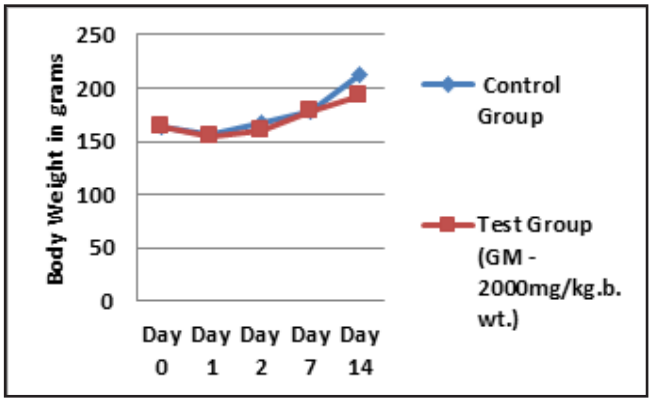

Figure 1. Changes in the body weight (in grams) of SD rats in acute oral toxicity study.

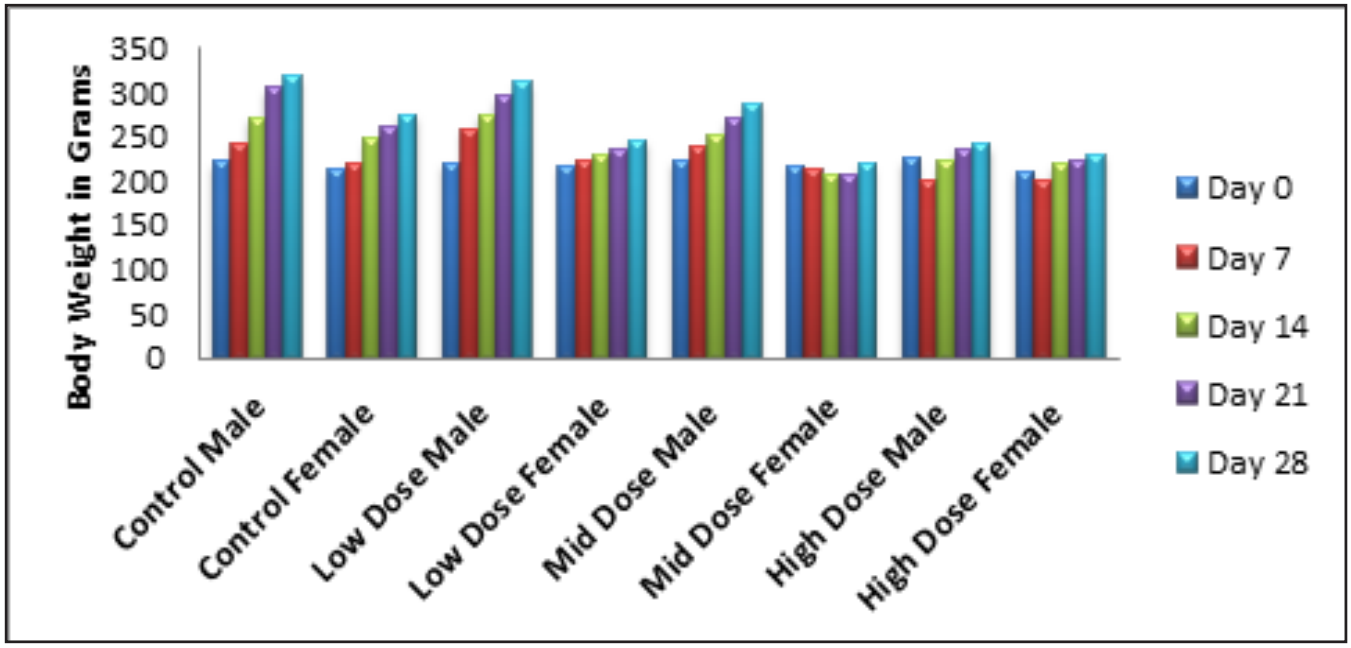

Figure 2. Changes in the body weight (in grams) of SD rats in repeated dose 28 day oral toxicity study.

animals (Figure 2). The hematological parameters such as total leucocyte count (WBC), differential (neutrophils, lymphocytes, eosinophils, basophils and monocytes) leucocyte count, reticulocyte count, total erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), platelet count and clotting time showed no significant changes between the control and treated groups on day 28 and 42 (Tables 1 and 2). Also the values were well within the clinical range of SD rats[9] of experimental groups.

No statistical significance in biochemical parameters such as glucose, total cholesterol, triglycerides, urea, creatinine, total protein, albumin, glutamyl pyruvate aminotransferase, alkaline phosphatase, and γ-glutamyl transferase were observed between control and treated grouped animals at day 28 and 42 (Tables 3 and 4). But, there is significant increase in ALT only in high dose group at day 28 and 42. Also, there is significant increase in Triglycerides in the high dose group (at 28th and 42nd days) and in both satellite groups (at 42nd day).

On observing serum electrolytes such as potassium, sodium, calcium and chloride, there were no significant changes between control and treated groups on day 28 and 42 (Table 5). No significant changes were observed in organ weights of Brain, heart, liver, kidneys, adrenals, spleen, epididymis, male sex glands, uterus with cervix and ovaries among all groups of animals. There were no macroscopic lesions were observed in the vital organs of control, low, mid and high dose groups. The histo-pathological study was carried out for the organs of animals in control and high dose groups and no abnormalities were found (Figure 3). For this reason, the histo-pathological study was not performed for the mild and mid dose group animals.

There were no treatment-related death, remarkable body weight changes and gross pathological findings observed in the experimental animals. Also, there were no significant changes in haematological, biochemical, and histopathological studies even at the dose of 400mg/kg b.wt. The No-Observed Adverse Effect Level (NOAEL) of GM was likely to be 400mg/kg b.w when administered orally to rats. Therefore, it was concluded that *GM* is safe for oral administration up to the dose of 400mg/kg b.wt.

Table 1. Effect of *GM* on hematological parameters in repeated dose 28 day oral toxicity study

Group / Treatment	Sex	WBC (10^3/uL)	RBC (10^6/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)	Clotting time (Sec)
28th Day										
I / Control (Water)	M	8.58 ± 2.27	4.47 ± **0.22**	15.18 ± **0.57**	21.68 ± **0.94**	48.62 ± **1.12**	33.98 ± **1.54**	70.00 ± **1.76**	441.80 ± **99.77**	101.80 ± 26.57
	F	7.16 ± **1.51**	4.48 ± **0.65**	14.32 ± **0.98**	22.44 ± **3.26**	50.16 ± **0.50**	32.20 ± **2.88**	64.38 ± **5.71**	443.80 ± **73.74**	123.40 ± 22.50
II / Low Dose (100mg/kg b.wt)	M	9.30 ± **1.87**	4.22 ± **0.12**	14.52 ± **0.64**	20.86 ± **0.77**	49.50 ± **0.74**	34.34 ± **1.01**	69.56 ± **1.30**	467.60 ± **56.77**	124.00 ± 42.36
	F	7.76 ± **1.20**	4.74 ± **0.75**	14.46 ± **1.06**	23.80 ± **3.75**	50.30 ± **0.84**	30.88 ± **3.79**	61.58 ± **7.76**	614.40 ± **85.90**	122.20 ± 44.39
III / Mid Dose (200mg/kg	M	8.46 ± **1.59**	4.52 ± **0.37**	14.38 ± **0.93**	21.76 ± **1.51**	48.50 ± **0.96**	31.98 ± **1.01**	66.10 ± **2.30**	531.80 ± **89.25**	142.20 ± 27.08
	F	8.54 ± **1.51**	5.93 ± **2.05**	13.50 ± **1.16**	25.38 ± **3.60**	49.20 ± **1.83**	31.82 ± **1.16**	65.62 ± **2.48**	577.40 ± **65.03**	139.60 ± 19.72
IV / High Dose (400mg/kg	M	9.32 ± **0.88**	3.92 ± **0.96**	14.44 ± **0.33**	19.46 ± **0.30**	45.14 ± **0.92**	33.36 ± **0.71**	74.14 ± **1.90**	329.80 ± **56.86**	204.20 ± 39.77
	F	6.90 ± **1.30**	4.61 ± **0.62**	14.44 ± **0.22**	21.64 ± **3.30**	46.92 ± **0.90**	31.68 ± **3.91**	67.78 ± **9.14**	478.60 ± **71.39**	188.40 ± 49.10
V / Control – Satellite (Water)	M	10.54 ± **1.98**	4.70 ± **0.26**	15.44 ± **0.80**	22.52 ± **1.28**	48.08 ± **0.98**	32.84 ± **0.30**	68.56 ± **1.43**	576.80 ± **83.42**	147.60 ± 19.45
	F	7.70 ± **0.89**	4.36 ± **0.15**	14.60 ± **0.30**	21.62 ± **0.75**	49.74 ± **0.35**	33.48 ± **0.67**	67.52 ± **1.65**	522.00 ± **84.14**	117.60 ± 44.73
VI / High Dose -Satellite (400mg/kg b.wt)	M	10.02 ± **1.00**	4.52 ± **0.39**	14.02 ± **0.45**	21.44 ± **0.70**	47.84 ± **1.92**	32.90 ± **1.10**	66.84 ± **1.18**	632.40 ± **11.50**	163.00 ± 7.81
	F	7.24 ± **0.72**	4.13 ± **0.19**	14.14 ± **0.62**	21.48 ± **1.08**	46.22 ± **1.60**	32.04 ± **0.92**	67.00 ± **1.50**	578.60 ± **38.72**	151.20 ± 26.71
				42nd Day						
V / Control – Satellite (Water)	M	9.04 ± **1.13**	4.26 ± **0.21**	14.82 ± **0.36**	19.90 ± **1.08**	46.90 ± **0.40**	34.88 ± **2.24**	74.64 ± **5.14**	402.00 ± **50.72**	172.60 ± 30.27
	F	6.58 ± **1.29**	4.16 ± **0.14**	14.52 ± **0.28**	20.62 ± **0.76**	48.74 ± **2.50**	34.90 ± **0.84**	70.44 ± **1.79**	423.40 ± **94.69**	218.60 ± 28.84
VI / High Dose -Satellite (400mg/kg b.wt)	M	6.94 ± 0.78	4.15 ± 0.18	13.96 ± 0.80	20.66 ± 1.28	44.76 ± 1.62	33.16 ± 1.05	72.44 ± 2.51	443.80 ± 46.45	185.40 ± 16.49
	F	6.06 ± 1.26	4.46 ± 0.21	14.68 ± 0.59	21.22 ± 1.25	46.44 ± 1.58	32.94 ± 0.55	70.20 ± 3.41	409.20 ± 69.72	209.40 ± 32.30

M – Male, F - Female, mg/kg b.wt - milligram/kilogram body weight, µL – Micro Litre, g/dL - Grams per Decilitre, pg - Picogram, fL - femto Litre, Sec - Seconds, % - Percent, Values expressed in mean ± SEM (n = 5).

Table 2. Effect of *GM* on differential leucocyte count and reticulocyte count in repeated dose 28 day oral toxicity study

Group / Treatment	Sex	Lymphocyte (%)	Monocytes (%)	Basophils (%)	Eosinophils (%)	Neutrophils (%)	Reticulocytes (%)
			28th Day				
I / Control	M	76.90 ± 4.10	4.20 ± 0.84	0.20 ± 0.45	2.00 ± 0.71	17.00 ± 2.74	0.80 ± 0.84
	F	79.40 ± 1.67	4.40 ± 0.89	0.20 ± 0.45	0.60 ± 0.55	15.40 ± 2.41	0.40 ± 0.55
II / Low Dose (100mg/kg b.wt)	M	79.00 ± 2.35	4.40 ± 1.14	0.00 ± 0.00	0.60 ± 0.89	16.00 ± 2.35	0.40 ± 0.89
	F	77.60 ± 3.78	4.40 ± 1.14	0.00 ± 0.00	1.20 ± 0.84	16.80 ± 2.59	0.40 ± 0.55
III / Mid Dose (200mg/kg b.wt)	M	76.80 ± 2.59	3.80 ± 0.84	0.20 ± 0.45	1.40 ± 1.67	17.80 ± 2.17	0.40 ± 0.55
	F	78.20 ± 2.49	4.20 ± 0.45	0.00 ± 0.00	1.20 ± 1.10	16.40 ± 1.52	0.60 ± 0.55
IV / High Dose (400mg/kg b.wt)	M	77.40 ± 3.21	4.00 ± 1.00	0.20 ± 0.45	0.80 ± 0.84	17.60 ± 2.61	0.20 ± 0.45
	F	76.40 ± 2.41	4.60 ± 0.89	0.00 ± 0.00	0.60 ± 0.55	18.40 ± 2.07	0.60 ± 0.89
V / Control - Satellite	M	78.00 ± 3.54	3.80 ± 1.30	0.20 ± 0.45	1.20 ± 1.30	16.80 ± 2.28	1.00 ± 1.00
	F	76.60 ± 3.78	4.60 ± 1.14	0.00 ± 0.00	1.00 ± 1.00	17.40 ± 3.44	0.40 ± 0.55
VI / High Dose - Satellite (400mg/kg b.wt)	M	73.60 ± 2.30	4.60 ± 1.14	0.00 ± 0.00	1.00 ± 0.71	15.60 ± 2.07	1.20 ± 0.84
	F	76.60 ± 3.78	4.60 ± 1.14	0.00 ± 0.00	1.00 ± 1.00	17.40 ± 3.44	0.40 ± 0.55
			42nd Day				
V / Control - Satellite	M	75.00 ± 2.00	3.60 ± 1.14	0.40 ± 0.55	1.20 ± 1.30	19.80 ± 1.92	1.00 ± 0.71
	F	76.00 ± 2.74	4.40 ± 1.14	0.20 ± 0.45	1.00 ± 1.00	18.40 ± 2.61	0.40 ± 0.55
VI / High Dose -Satellite (400mg/kg b.wt)	M	75.20 ± 2.39	4.00 ± 1.87	0.20 ± 0.45	0.60 ± 0.55	20.00 ± 3.74	1.20 ± 0.84
	F	73.60 ± 2.88	5.00 ± 1.00	0.00 ± 0.00	1.20 ± 0.84	20.20 ± 2.17	1.20 ± 0.84

M - Male, F - Female, mg/kg b.wt - milligram/kilogram body weight, % - percent, values expressed in mean ± SEM (n = 5).

Table 3. Effect of *GM* on liver function test of SD rats in repeated dose 28 day oral toxicity study

Group/Treatment	Sex	Albumin (G/Dl)	ALP-AMP (U/L)	ALT (U/L)	Total Bilirubin (Mg/Dl)	Total Protein (G/Dl)
28th Day						
I/Control	M	3.51 ± 0.11	50.80 ± 7.19	31.80 ± 5.07	1.40 ± 0.31	7.68 ± 0.25
	F	3.54 ± 0.26	25.80 ± 3.70	28.80 ± 5.54	2.02 ± 0.72	7.41 ± 0.55
II/Low Dose (100mg/kg b.wt)	M	3.41 ± 0.39	74.60 ± 25.23	39.60 ± 10.11	1.64 ± 0.11	7.93 ± 0.47
	F	3.61 ± 0.16	36.00 ± 2.92	26.60 ± 5.22	1.82 ± 0.38	8.15 ± 0.40
III/Mid Dose (200mg/kg b.wt)	M	3.53 ± 0.18	115.00 ± 57.48	58.00 ± 16.76	15.0 ± 0.39	7.79 ± 0.66
	F	3.67 ± 0.14	108.40 ± 45.92	47.80 ± 20.64	1.82 ± 0.75	7.97 ± 0.56
IV/High Dose (400mg/kg b.wt)	M	3.21 ± 0.18	137.00 ± 62.69	152.00 ± 133.47	0.72 ± 0.37	7.69 ± 0.47
	F	3.35 ± 0.14	91.80 ± 31.20	142.20 ± 94.12	0.62 ± 0.23	7.52 ± 0.29
V/Control - Satellite	M	3.50 ± 0.15	65.00 ± 7.48	33.00 ± 5.87	2.20 ± 0.57	8.37 ± 0.48
	F	3.57 ± 0.08	34.00 ± 9.72	28.80 ± 5.12	1.78 ± 0.13	8.14 ± 0.38
VI/High Dose -Satellite (400mg/kg b.wt)	M	3.44 ± 0.24	38.80 ± 9.76	23.40 ± 2.41	1.30 ± 0.16	7.27 ± 0.48
	F	3.92 ± 0.20	53.20 ± 9.71	29.60 ± 2.88	1.42 ± 0.22	7.05 ± 0.13
V/Control - Satellite	M	3.28 ± 0.09	111.40 ± 13.46	47.60 ± 6.47	0.58 ± 0.11	7.28 ± 0.20
	F	3.53 ± 0.10	61.60 ± 17.27	44.00 ± 5.96	0.72 ± 0.19	7.25 ± 0.20
VI/High Dose -Satellite (400mg/kg b.wt)	M	3.17 ± 0.29	87.60 ± 13.78	163.60 ± 15.73	0.54 ± 0.11	6.69 ± 0.77
	F	3.48 ± 0.13	105.20 ± 23.54	191.00 ± 79.38	0.66 ± 0.11	7.79 ± 0.23

M - Male, F - Female, mg/kg b.wt - milligram/kilogram body weight; g/dL - grams per Decilitre, mg/dL - mgms per decilitre, values expressed in mean ± SEM (n=5); *indicates p value < 0.05 vs. control group

Table 4. Effect of *GM* on biochemical parameters of SD rats in repeated dose 28 day oral toxicity study

Group / Treatment	Sex	Cholesterol (Mg/Dl)	TGL (Mg/Dl)	Urea (Mg/Dl)	Creatinine (Mg/Dl)	g-GT (U/L)	Glu (Mg/Dl)
28th Day							
I/Control	M	42.60 ± 3.65	28.00 ± 6.93	37.00 ± 5.39	0.40 ± 0.03	0.80 ± 0.84	38.20 ± 7.29
	F	46.40 ± 6.80	19.80 ± 4.32	44.20 ± 5.76	0.51 ± 0.07	0.40 ± 0.89	53.20 ± 15.93
II/Low Dose (100mg/kg b.wt)	M	46.00 ± 5.43	31.00 ± 14.75	40.40 ± 3.85	0.43 ± 0.03	0.40 ± 0.55	41.40 ± 19.35
	F	56.40 ± 8.05	27.20 ± 9.60	43.80 ± 7.09	0.49 ± 0.06	0.20 ± 0.45	36.00 ± 10.44
III/Mid Dose (200mg/kg b.wt)	M	61.20 ± 21.37	49.20 ± 13.20	42.20 ± 16.16	0.50 ± 0.11	1.80 ± 4.02	40.20 ± 10.16
	F	97.00 ± 20.48	47.00 ± 31.99	63.00 ± 39.80	0.52 ± 0.12	0.00 ± 0.00	67.42 ± 55.30
IV/High Dose (400mg/kg b.wt)	M	112.20 ± 34.20	316.20 ± 170.35	37.80 ± 4.66	0.65 ± 0.03	5.20 ± 6.14	76.15 ± 20.62
	F	113.80 ± 15.32	326.20 ± 109.48	40.80 ± 3.96	0.64 ± 0.10	8.60 ± 12.56	69.15 ± 8.47
V/Control - Satellite	M	49.20 ± 8.44	33.00 ± 8.12	30.60 ± 4.51	0.46 ± 0.06	0.00 ± 0.00	53.80 ± 11.90
	F	54.40 ± 8.68	22.20 ± 8.96	30.60 ± 11.01	0.46 ± 0.01	0.20 ± 0.45	50.60 ± 7.77
VI/High Dose -Satellite (400mg/kg b.wt)	M	47.60 ± 5.59	27.20 ± 3.19	28.20 ± 4.66	0.50 ± 0.07	0.60 ± 0.55	50.40 ± 4.28
	F	46.60 ± 5.68	41.40 ± 4.72	27.60 ± 3.05	0.54 ± 0.08	0.40 ± 0.55	58.80 ± 7.89
42nd Day							
V/Control - Satellite	M	76.60 ± 12.46	379.00 ± 129.86	37.60 ± 3.78	0.58 ± 0.11	2.60 ± 0.89	73.40 ± 13.36
	F	86.20 ± 14.02	318.80 ± 28.89	40.60 ± 2.07	0.74 ± 0.04	2.00 ± 1.00	81.68 ± 25.57
VI/High Dose -Satellite (400mg/kg b.wt)	M	157.80 ± 16.48	390.00 ± 50.40	39.40 ± 2.07	0.65 ± 0.05	2.60 ± 1.14	67.44 ± 6.62
	F	149.40 ± 33.93	384.00 ± 61.37	39.00 ± 5.79	0.81 ± 0.05	2.60 ± 2.51	71.73 ± 19.30

M - Male, F - Female, mg/kg b. wt - milligram/kilogram body weight, g/dL - grams per Decilitre, mg/dL - milligrams per decilitre, values expressed in mean ± SEM (n=5), * indicates p value < 0.05 Vs control group

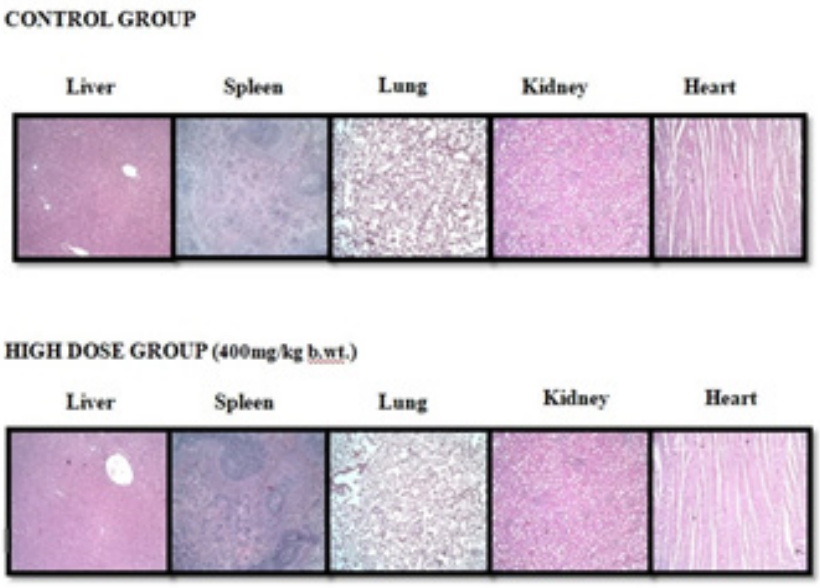

Figure 3. Histo-patholoIgical observations of vital organs of SD rats after repeated dose 28 day oral toxicity study.

Table 5. Effect of *GM* on serum electrolytes of SD rats in repeated dose 28 day oral toxicity study

Group	Treatment	Sex	K (mmol/L)	Na (mmol/L)	Cl (mmol/L)	Ca (mmol/L)	Total Ca (mmol/L)
Day 28							
I	Control	M	5.36±0.32	147.41±5.15	94.66±1.97	1.19±0.09	2.38±0.18
		F	5.57±0.33	154.14±7.11	92.16±2.99	1.22±0.07	2.44±0.13
II	Low Dose (100mg/kg b.wt)	M	4.57±0.79	133.93±8.60	91.74±10.31	1.15±0.12	2.30±0.25
		F	4.92±0.46	139.70±4.82	91.31±6.68	1.21±0.10	2.43±0.21
III	Mid Dose (200mg/kg b.wt)	M	5.67±0.97	142.75±3.92	102.84±6.72	1.24±0.03	2.48±0.06
		F	5.48±0.65	143.03±2.78	97.06±6.07	1.25±0.04	2.53±0.05
IV	High Dose (400mg/kg b.wt)	M	4.65±0.43	141.01±0.67	107.46±0.29	1.24±0.02	2.48±0.04
		F	4.26±0.21	140.47±1.02	107.63±1.26	1.25±0.05	2.50±0.10
V	Control - Satellite	M	6.68±0.70	140.98±3.54	82.30±9.86	1.18±0.08	2.36±0.15
		F	5.56±0.93	138.28±2.14	101.64±5.25	1.20±0.08	2.40±0.16
VI	High Dose -Satellite (400mg/kg b.wt)	M	4.87±0.22	138.93±1.71	102.79±2.35	1.14±0.08	2.26±0.15
		F	4.53±0.28	132.89±3.80	104.45±3.27	1.44±0.28	2.41±0.17
Day 42							
V	Control - Satellite	M	4.79±0.16	138.27±0.49	104.03±0.61	1.12±0.02	2.24±0.05
		F	4.27±0.28	138.93±1.15	104.76±1.23	1.16±0.04	2.32±0.08
VI	High Dose -Satellite (400mg/kg b.wt)	M	4.81±0.10	138.26±2.72	104.72±2.81	1.33±0.15	2.63±0.09
		F	4.23±0.20	140.56±1.37	109.31±0.70	1.34±0.08	2.61±0.09

M - Male, F - Female, mg/kg b. wt - milligram/kilogram body weight, mmol/L - millimolar per litre, values expressed in mean ± SEM (n=5), *, ** - indicates p value < 0.05 and 0.01, respectively.

4. Discussion

Traditional medicines that are proven with quality, safety, and efficacy can be contributed to achieve WHO's goal of ensuring health care access to all people in this world. They are the major source of health care and on occasions, they are the only accessible source to millions of people[10]. *Siddha* system of medicine is one of the traditional medicines of India, which is customary in Southern parts of India, mainly in Tamil Nadu. In order to guarantee the safe usage of traditional medicines, their application on humans in the olden days and toxic evaluation through scientific methods, both need to be considered[11]. *GM* is one of the most promising *Siddha* drugs being used in the clinical practice for the treatment of psoriasis. However, the scientific validation of its safety is not yet established. In addition to its historical usage on humans, it is also mandate to prove its safety through scientific methods. This present study investigates the safety of *GM* in SD rat models through acute and sub-acute toxicity studies.

Since *GM* is being used clinically for many years, the limit test can be performed at the highest starting dose level (2000 mg/kg b.wt.) according to OECD test guideline 423[6]. No changes related to toxicity and no mortality were observed in *GM*-treated animals. Hence, the LD50 value of *GM* was found to be greater than 2000mg/kg b.wt. Regarding the Globally Harmonised System of Classification and Labelling of chemicals, *GM* can be classified as Category – 5. This directly indicates the safety of *GM* when used as a single dose orally.

According to OECD test guideline 407[8], 28 days repeated oral toxicity study was conducted in SD rats (both male and female) with 100, 200, and 400 mg/kg orally for 28 consecutive days. In addition to these low, mid, and high dose groups, control group and two other satellite groups were maintained. No death was observed during the observation period. The gradual increase in body weight (Figure 2) along with no significant change in food and water consumption implies that *GM* did not adversely affect the basal metabolic processes of all the experimented animals when compared to the control group animals. Biochemical and hematological data indicates the toxic effect induced by the drugs[12]. The hematological parameters are a sensitive index to give clue about the diseased conditions in both animals and humans[13]. From Tables 1 and 2, these parameters show no significant changes in experimental grouped animals indicating that *GM* does not affect the blood cells. Moreover, elevated liver enzymes like SGOT and SGPT clearly states the toxic nature of drug as it is an indicator of damaged liver parenchyma[14]. Since, the parameters of Liver Function Test (Table 3) are also within the clinical referral range of SD rats, *GM* is safe for liver parenchymal cells. The rise of serum ALT concentration is a sensitive but not precise measure of hepatocellular injury. Hence, the elevation in ALT levels of high dose animal group cannot be taken as serious indicator, as other liver enzymes are all within the normal range[15]. Similar to biochemical and hematological analysis, the serum electrolyte levels (Table 5) also shows no test drug related abnormality. No significant changes were found in relative organ weights (Table not shown), gross necropsy and histopathology of both control and treated group animals (Figure 3). These results suggest that *GM* is non-toxic, and it is safe for human consumption.

5. Conclusion

In this present study, regarding to the Globally Harmonised System of Classification and Labelling of chemicals, *GM* can be classified as Category-5. Based on the results of 28 day Repeated Oral Toxicity study, it is evident that GM is non-toxic up to the dose of 400mg/kg b.wt. and it can be used for safe human consumption. This study substantiates the safety of *GM*, at least a part. Further Sub-chronic and Chronic toxicity studies need to be carried out to validate its safety in long-term administration.

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