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ACUTE AND SUB ACUTE TOXICITY STUDIES OF HERBO-METALLIC FORMULATION VANGA CHENDURAM

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ABSTRACT

Siddha medicine is the traditional system of heeling and it's based on combination of ancient medical practices and disciplines as well as alchemy and mysticism. In this study author aim to evaluate the acute and sub-acute toxicity study of trial formulation Vangachenduram. To substantiate this trial formulation for skin diseases as quoted in siddha text, without any adverse effects and to prove this drug to be non-toxic as per OECD guidelines 423. As per the study conducted in Wistar rats, the biochemical, hematological, lipid profile, histopathological studies are evaluated. The result proves that this trial formulation is non-toxic.

Keywords: *Vangachenduram*, acute toxicity, sub-acute toxicity, Wistar rats.

1 INTRODUCTION:

Unlike other system of medicine, which gives top most priority to herbal treatment, siddha medicine gives conjunctive use of plants, metals, and minerals. In due course of time herbo metallic preparation occupy a significant seat in traditional system of medicine. While such preparation are held to be safe, effective in minute doses, when prepared and used following specific guidelines. One such effective safe herbo metallic formulation is vangachenduram. It constitutes Vangam (lead), Aloe vera, Vediuppu. Vangam was a heavy metal but it can be safe and effective when its purified and used in perfect procedure.

Aim and objective: To study the acute and sub acute toxicity of these trial formulation Vangachenduram.

2. MATERIAL AND METHODS:

2.1 Collections of drugs

The drugs were collected from reputed raw drug shop in Madurai. These drugs were analyzed and authenticated by Government Siddha Medical College, Palayamkottai.

2.2 Method of preparation of Vangachenduram

Purified vangam is taken and it is melted in frying pan, *krasam* is given little by little with a mixture of *shotrukathalaiver (Roots of Aloe Vera) and chirukeeraiver (Roots of Amaranthus tricolor)* cut into fine bits, to which Vediuppu nicely powdered is added and properly mixed. Frying is done with an iron vessel, under kadagni from the beginning. Frying is continued for 3 days until the medicine is free from moisture & metal particles become red in colour. The longer we fry over intense fire, the better the qualities&colour of chenduram. Frying is to be continued even after

the krasam powder is exhausted. At times it becomes snuff colored or of rose colour. On being cooled it is taken out, powdered and bottled up.

2.3 Selection of animal species Healthy young adult female nulliparous or non-pregnant Wistar albino rats of 8-12 weeks, weighing 150-250 grams were obtained from the King Institute of Preventive medicine, Guindy, Chennai. The rats were used after obtaining Institutional Animal Ethical Committee clearance bearing the-**IAEC approval No. IAEC/XLIV/34/CLBMCP/2014**

They were kept in C.L.BaidMetha College of pharmacy, Thoraipakkam, Chennai,

2.4 Animal selection and maintenance:

Species	:	Rat
Strain	:	Wistar albino
Sex	:	Female (Nulliparous / non pregnant)
Age/ Weight at start of test	:	8 - 12 wk, 150 - 250 g
Acclimatization Period	:	7 days prior to dosing
Housing	:	Individually in polypropylene cages
Husbandry	:	12-h light/12-h dark artificial photoperiod
Room Temperature	:	22°C (±3°)
Relative Humidity	:	30-70%
Food (SaiMeera Foods, Bangalore)	:	Rodent pelleted feed
Water	:	RO purified water
Identification	:	Rats will be kept in individual cages and numbered
Duration of Study	:	48 hrs
Evaluation	:	14 Days

2.5 Preparation of rats: The rats were randomly selected, marked to permit individual identification, and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

2.6 Preparation of doses

a) Drug Stock solution: The powdered-form of *Drug* was mixed uniformly in 2% CMC and made into uniform suspension to achieve 200mg/ml as main stock solution and used in this study and was found suitable for dose accuracy.

b) Justification for choice of vehicle: The vehicle was selected as per the standard guideline which was pharmacologically inert and easy in employing new drug development and evaluation technique.

c) Administration of doses

Duration of exposure to test drug : Single dose

Route of administration : Oral by gavage using stomach tube

Before drug administration, rats are fasted overnight but not water. After administration of the drug, food was withheld for a further 3-4 hrs.

d) No. of rats and dose levels: Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight was carried out with 6 rats (3 rats per step).

3. OBSERVATION: The rats were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general be-

haviour and other physiological activities and daily thereafter, for a total of 14 days.All observations were systematically recorded with individual records being maintained for each animal.

a. **Mortality:** Rats were observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. **Body weight:** Individual weight of rats was determined before the test substance was administered and 1, 2, 7 and 14 days of the study at least weekly thereafter. Weight changes was calculated and recorded. At the end of the test surviving rats were weighed and humanely killed.

c. **Cage-side observation:** These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, vasomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

II. Repeated dose 28-day oral toxicity study of VANGA CHENDURAM on Wistar rats (OECD – 407 guidelines)

1. Principle of the test: The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. A 28 day study provides information on the effects of repeated oral exposure and can indicate the need for further longer term studies. It can also provide information on the selection of concentrations for longer term studies. The data derived from using the TG should allow for the characterization of the test substance toxicity, for an indication of the dose response relationship and the determination of the No-Observed Adverse Effect Level (NOAEL) (OECD Guidelines 407, Adopted in 2008).

2.Randomization, Numbering and Grouping of Animals: Ten female rats were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment.

a. **Justification for Dose Selection:**The results of acute toxicity studies in wistar rats indicated vangachenduram was non toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. In the literature, therapeutic dosage for vangachenduram in human is mentioned as 130 mg. On the basis of body surface area ratio between rat and human, the doses selected for the study were 100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

b. **Preparation and administration of dose:***Vangamchenduram*at two doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100 and 200 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral gavage, once daily for 28 consecutive days. Before drug administration, rats were fasted overnight but not water. After administration of the drug, food was withheld for a further 3-4 hrs.

3. OBSERVATION Experimental animals were kept under observation throughout

the course of study for the following parameters and were assessed as follows,

a) **Body Weight**Weight of each Wistar rat was recorded on day 0, at weekly intervals throughout the course of study and at termination of 28th day to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

b) **Clinical signs:** All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms were recorded.

c) **Mortality:** All animals were observed twice daily for mortality during entire course of study.

d) **Functional Observations:**At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

e) **Laboratory Investigations**Following laboratory investigations were carried out on day 29 in animals'fasted overnight blood samples. Blood samples were collected from orbital sinus using sodium heparin (200 IU/ml) for blood chemistry and potassium EDTA (1.5 mg/ml) for haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

f) **Haematological Investigations:** Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

g) **Biochemical Investigations:**Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods.Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

h) Necropsy: All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

i. **Histopathology:** Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hour and washed in running water for 24 hour. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.The organs including kidney, liver and spleen of the animals were preserved and they were subjected to histopathological examination.

4. Results of Acute oral toxicity in rats

a) Dose finding experiment and its behavioral Signs of Toxicity for MLM

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

1. Alertness - Present
2. Aggressiveness- Absent
3. Pile erection- Present
4. Grooming - Present
5. Gripping- Absent
6. Touch Response - Absent
7. Decreased Motor Activity-Absent
8. Tremors- Absent
9. Convulsions- Absent
10. Muscle Spasm- Absent
11. Catatonia - Absent
12. Muscle relaxant- Absent
13. Hypnosis - Absent
14. Analgesia - Absent s
15. Lacrimation- Absent
16. Exophthalmos - Absent

b)Body weight (g) changes of rats when exposed to Sample

Dose (mg/kg/day)	***Days***				
	0	**7**	**14**	**21**	**28**
Control	130.37±1.14	131.14±1.06	135.21±1.12	136.21±1.12	142.32±1.24
100	138.27□3.14	140.72□6.28	142.5 □4.4	145.43±2.14	148.8 □2.42
200	140.5 □3.24	158.45□5.34	152.25□3.22	156.64□3.18	160.5 □4.14

*Values are expressed as mean ± S.E.M; n=06; *P<0.05, **P<0.01, ***P<0.001 vs control.*

The above results showed that the body weight did not differ and remained within the normal limits.

c) Effect of sample on organ weight in rats

Organ	**Control**	**100 mg/kg**	**200 mg/kg**
Liver (g)	4.14□0.14	4.8±0.45	5.79±0.3
	0.64 0.07	0.56±0.07	0.76±0.04
Kidney (g)	0.80□0.05	0.48±0.07	0.73±0.05

The above results showed that all parameters remained within normal limits.The rats did not reveal any observable signs of central nervous system, any behavioural signs and mortality.

d) Results of Hematological parameters:

Parameter	Control	100mg/kg	200mg/kg
Heamoglobin	12.5 ± 0.16	14.6±0.2	16.2±0.4
PCV (%)	38.2 ± 2.04	45.3±2.2	48.4±2.6
RBC(x10^{6}/mm^3)	14.6 ± 1.18	5.5±0.6	6.2±1.10
WBC (x 10^3/mm^3)	10.12 ± 1.2	5.8±1.02	6.7±1.2
Platelet(x10^3/mm^3	900± 48.8	360± 22.8	420± 14.8
Polymorphs (%)	34± 00.14	39± 12.02	42± 1.8
Lymphocytes (%)	75± 24.8	57± 10.5	59± 8.4
Eosinophil (%)	06± 0.08	03± 1.2	04± 1.02
Monocytes (%)	0.6± 0.08	0.1± 0.02	0.2± 1.2
Basophills(%)	00	00	00

e)Bio chemical parameters:

BIOCHEMISTRY	Units	Normal value	Result
Blood sugar (Random)	mg/dl	90-140	40
Serum creatinine	mg/dl	0.3 – 1.4	0.5
Serum Protein	g/dl	6.0 – 8.4	7.1
Serum albumin	g/dl	3.8 -5.0	4.3
SGPT (ALT)	IU/L	Upto 40	54
SGOT (AST)	IU/L	Upto 40	165
Blood urea (BUN)	mg/dl	08 -20	17

f)Lipid profile:

lipids	**Units**	**Normal values**	**Drug treated**
Cholesterol- Total	**mg/dl**	**150 -200**	**95**
Triglycerides	**mg/dl**	**50 -150**	**86**
HDL cholesterol	**mg/dl**	**35 - 75**	**26**
LDL cholesterol	**mg/dl**	**80 - 100**	**51.80**
VLDL cholesterol	**mg/dl**	**10 - 30**	**17.20**
Cholesterol/HDL Ratio		**< 5**	**3.65**

5. CONCLUSION

The toxicological study of vangachenduram clearly states that there is know toxicity and adverse effects. It is evident from their histopathological study, haematological parameters, and biochemical analysis and body weight on 1st day and 28th day.

REFERANCES:

1. *Siddha Research pharmacopeia*

2. VOGELS-The textbook of basic animal experimental procedure.

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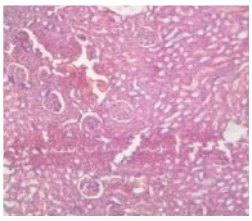
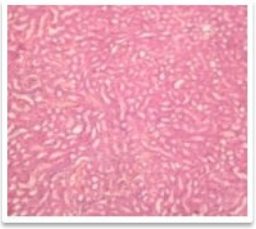
g) HISTOPATHOLOGY REPORT / MICROSCOPIC EXAMINATION

1. KIDNEY

CONTROL **DRUG TREATED**



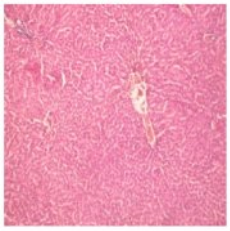
Normal glomerulus and renal tubules



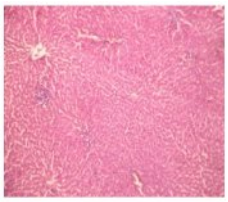
Normal glomerulus and renal Tubules

2. LIVER

Control liver **Drug treated liver**



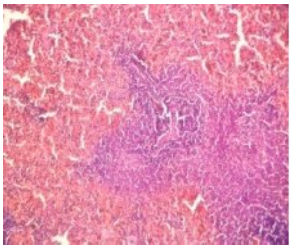
NORMAL

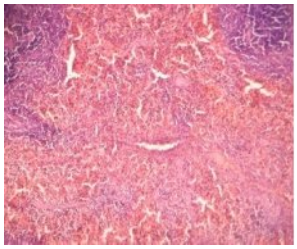


NORMAL

3. SPLEEN

Control SPLEEN **Drug treated SPLEENS**





Intra parenchymal hemosiderin laden macrophages.