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Acute and Sub-Acute Toxicity Study of Siddha Herbo-Mineral Formulation "Panchamuga Chendhuram" in Experimental Rats

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***S. RAJALKSHMI[1], M. MOHAMED MUSTHAFA[2]**

1. PG Scholar, Dept. of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai, Tamilnadu.

2. Assist. Prof., Dept. of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai, Tamilnadu, India.

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ABSTRACT

Background: Panchamuga chendhuram (PMC) is a Siddha Herbo-mineral formulation indicated for all skin diseases in classical Siddha literature Pullipani vaidhyam-500. It contains 5 ingredients *Rasam* (Mercury) (Hg), *Gandhagam* (Sulphur) (s), *Lingam* (Mercuric II sulfide) (Hgs), *Thaalagam* (Arsenic trisulphidium) (AS_2S_3), *Veeram* (Mercuric chloride) ($Hgcl_2$). Since heavy metal toxicity being a major issue in Herbo-mineral formulations, the present preclinical study aims to carry out safety and toxicity of PMC (IAEC NO.: IAEC/XLII/01/CLBMCP/2014 dated 22.01.2014). **Materials and Methods:** Adult of both sexes of Wistar albino rats weighing 150-250gm of 8-12 weeks were used. Acute and Sub-acute toxicity were carried out as per OECD guidelines 423 and 407. Hematological parameters, Biochemical parameters, Histopathological study were performed for all animals. **Conclusion:** The study concludes that on oral administration of 20mg/kg of body weight of PMC to Wistar albino rats, there was no change in behaviour movements and no characteristic clinical sign of toxicity or mortality observed. There was also no significant change in the liver and kidney function test and hematological parameters. Histopathalogical evidence of gross pathological changes were not showed in the vital organs.

INTRODUCTION

In recent years, complementary and alternative medicine (CAM) has increased globally for the treatment of many chronic ailments [1]. However, there is always fear about their safety, efficacy, toxicity of CAM therapies. **"PANCHAMUGA CHENDHURAM"** is one of the Herbo-mineral formulation mentioned in classical Siddha literature Pulippani vaithyam-500 indicated for all skin diseases is selected for this study[2]. It contains 5 ingredients *Rasam* (Mercury) (Hg), *Gandhagam* (Sulphur) (S), *Lingam* (Mercuric II sulfide) (HgS), *Thalagam* (Arsenic trisulphidium) (AS_2S_3), *Veeram* (Mercuric chloride) ($HgCl_2$).

Mercury is used to treat syphilis, gonorrhea, leprosy. It has tonic, alternative, antiseptic actions. It increases number of RBCs[3]. Sulphur regulates cell division. It has antioxidant effects. It is an inflammatory inhibitor. It is used in chronic skin diseases, in advanced leprosy[3]. Arsenic trisulphidium is used in chronic skin diseases such as eczema, psoriasis, leprosy etc.[3]. Mercury (II) sulfide is used in eczema, leprosy, bronchial asthma, arthritis[4]. Mercurous Chloride has broad spectrum antimicrobial activity. It has good antibacterial effect on a variety of Gram-positive and Gram-negative bacteria and pathogenic skin fungi[5]. Hence, the present investigation was undertaken to assess the safety profile of Siddha drug PMC used to treat skin diseases in animal models using OECD guidelines. This is important because incomplete knowledge about the toxicity profile of a drug will lead to severe adverse effect [6].

MATERIALS AND METHODS

Sources of Drug

The raw drugs were procured from the raw drug shop R. N. Rajan and Co, Chennai. After proper authentication by the Pharmacognosist, Siddha Central Research Institute, Arumbakkam, Chennai the preparation was made.

Panchamuga Chendhuram preparation

Raw drugs were purified according to the classical procedure mentioned in text Siddha Materia medica, Mineral and Animal kingdom sections[4].

Materials used

- Purified *Rasam* (Hydragyrum) 100g,
- Purified *Gandhagam* (Sulphur) 100g,
- Purified *Thaalagam* (Arsenic trisulphidium) 100g,
- Purified *Lingam* (Mercury II sulfide) 100g,
- Purified *Veeram* (Mercuric chloride) 100g,
- Piper betel leaf juice Q.S.

Equipments used

Small mud pot (*Satti*), Big mud pot (*Satti*), Cloth pieces, Mortar with pestle.

Procedure

P. *Gandhagam* was grounded with vettrillai charu (piper betel juice), P. *Rasam* was added to it and grounded well. To this mixture P. *Thaalagam* and P. *Lingam* was added. Finally P. *Veeram* was added and grounded well for 1 day.

Then small *villais* were made, and these *villais* was kept inside the small mud pot. The small mud pot was covered with small mud plate and sealed with 7 mud clothes. The big mud pot was filled with sand and small mud pot was placed inside it. Then the big mud pot was covered with big mud plate and sealed with 7 mud clothes. It is heated by small flame (Deepaagni) - 6hrs, moderate flame (Kamalaagni) - 6hrs, high flame (Kaadaagni) - 9hrs. After self cooling, seal was opened and the product was again subjected to grinding for 1 day. The final product (PMC) was weighed and stored in an air tight container.

Precautions

1. While grinding *Veeram* gloves were weared.

2. Gloves were weared while making *Villai*.

TOXICITY STUDY

The experimental protocol was permitted by the Institutional Ethical Committee (IAEC) under CPCSEA, approval no: IAEC/XLII/01/CLBMCP/2014 dated 22.01.2014 of C.L Baid Metha College of Pharmacy, Thurai Pakkam, Chennai, Tamilnadu.

Acute toxicity study

Three female nulliparous and non-pregnant rats were used for acute oral toxicity study according to Organization for Economic Cooperation Development (OECD) guidelines 423 [7]. PMC was administered orally 10mg/kg body weight of different groups of rats and absorbed for toxicological study. The animals were observed individually after dosing the first 30mins, periodically during the first 24h, with special attention given during the first 4h, and daily thereafter, for 14 days. Observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defaecation), and central nervous system (drowsiness, gait, tremors, and convulsions) changes respectively **(Table1)**. Mortality, if any, was determined over a period of 2 weeks.

Table 1: Observations in toxicity studies

	Observation
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion (Limb paralysis)	Absence of sign (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Tremor	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defaecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Sub Acute Oral Toxicity

This sub-chronic oral toxicity study was carried out according to OECD guideline 407[8,9]. In this study, the animals were divided into three groups of each 6 animals (3 males and 3 females) and

treated with low (10mg/kg of body weight) and high dose (20mg/kg of body weight) levels to be administered for 28 days. Group 1 received 0.025% CMC in water and served as control, Groups 2 and 3 received 10mg/kg and 20mg/kg PMC (suspended in 0.025% CMC solution) body weight orally, respectively. The drug was administered daily for 28 days at the same time and observed at least twice for morbidity and mortality. Body weights and food consumption of the animals were observed weekly.

Test substance suspensions were freshly prepared every day for 28 days. Control animals were administered vehicle only.

GROUP 1 - Control, received 0.025% CMC [carboxy methyl cellulose].
GROUP 2 - Treated with low dose of PMC 10mg/kg of body weight.
GROUP 3 - Treated with high dose of PMC 20mg/kg of body weight.

Hematological and Blood Biochemical Analysis

On the 29th day, of the sub-acute oral toxicity, over a period of fasting, the rats were anesthetized with ether and blood sample for hematological and biochemical analysis were collected by cardiac puncture method into tubes with and without Ethylene diamine tetra acetate (EDTA), respectively. Hematological parameter observed and recorded **(Table 2).** Biochemical parameter such as serum cholesterol, Triglyceride, Total protein, SGOT and SGPT, Alkaline phosphate also recorded **(Table 3)** [10, 11,12, 13,14].

Histopathological Study

Animals in the study were also subjected to a full, detailed gross necropsy. The positions, shapes, sizes and colors of internal organs (heart, kidney, brain and liver) were also recorded. The liver, heart, kidney, brain samples from each group were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections of thickness of about 5 µm were cut and stained with hematoxylin and eosin for Histopathological investigation (Fig. 1) [15].

Statistical Analysis

The data were expressed as means ± standard deviation (S.D). Student's paired 't'test was applied to analyze the results. P <0.01 was considered significant.

Table 2: Effect of PMC on Hematological parameters (Mean, S.E. of 3 animals)

Parameter	Control	10mg/kg	20mg/kg
RBC (x 10^6/mm^3)	7.51 ± 0.16	6.46±0.32	7.4±1.46
PCV (%)	48.2 ± 1.3	43.3±2.12	48.2±1.62
Hb (%)	15.6 ± 0.19	14.8±2.46	14.9±3.6
WBC (x 10^3/mm^3)	10.12 ± 1.2	11.5±1.6	11.7±2.1
Neutrophils (%)	22 ± 4	26.46±5.3	34.2±4.2
Mononuclear cells (%)	76 ± 2	72.4±3.8	60.4±2.3**
Eosinophils (%)	2.4 ± 0.6	2.2±0.28	1.6±0.06
Platelets (x 10^3/mm^3)	423.2 ± 48.8	424.56±16.4	446.14±32.6

Table 3: Effect of PMC on liver and renal parameters (Mean, S.E of 3 animals)

Parameters	Control	10mg/kg	20 mg/kg
Protein (g/dl)	8.62 ± 1.3	6.9±0.94	8.6±0.56
Albumin (g/dl)	4.8 ± 0.6	4.4±0.34	3.7±0.54
BUN (mg/dl)	19.2 ± 1.2	21.2±1.4	24.81±4.6
Urea (mg/dl)	64.24 ± 3.11	66.42±5.6	68.3±4.2
Creatinine (mg/dl)	0.82 ± 0.16	0.46±0.02*	0.72±0.06
Total Cholesterol (mg/dl)	91.24 ± 1.35	96.5±7.9	117.14±8.2*
Triglycerides (mg/dl)	50.15 ± 3.21	59.3±4.4	69.14±3.12**
Glucose (mg/dl)	110.16 ± 8.62	97.67±5.18	117.8±5.26

Total Bilirubin (mg/dl)	0.205 ± 0.04	0.242±0.06	0.533±0.07**
SGOT (U/L)	73 ± 2.4	53.2±4.4*	66.8±2.84
SGPT (U/L)	28.4 ± 1.2	33.6±3.46	59.7±6.69**
Alkaline phosphatase (U/L)	102.4 ± 3.6	96.7±6.6	103.6±4.9
Sodium (mEq/L)	138.12 ± 3.14	112.7±2.6	136.42±7.32
Potassium (mEq/L)	7.2 ± 1.34	3.26±0.18	5.42±0.28

PMC – SUBACUTE TOXICITY HISTOPATHOLOGICAL SLIDES

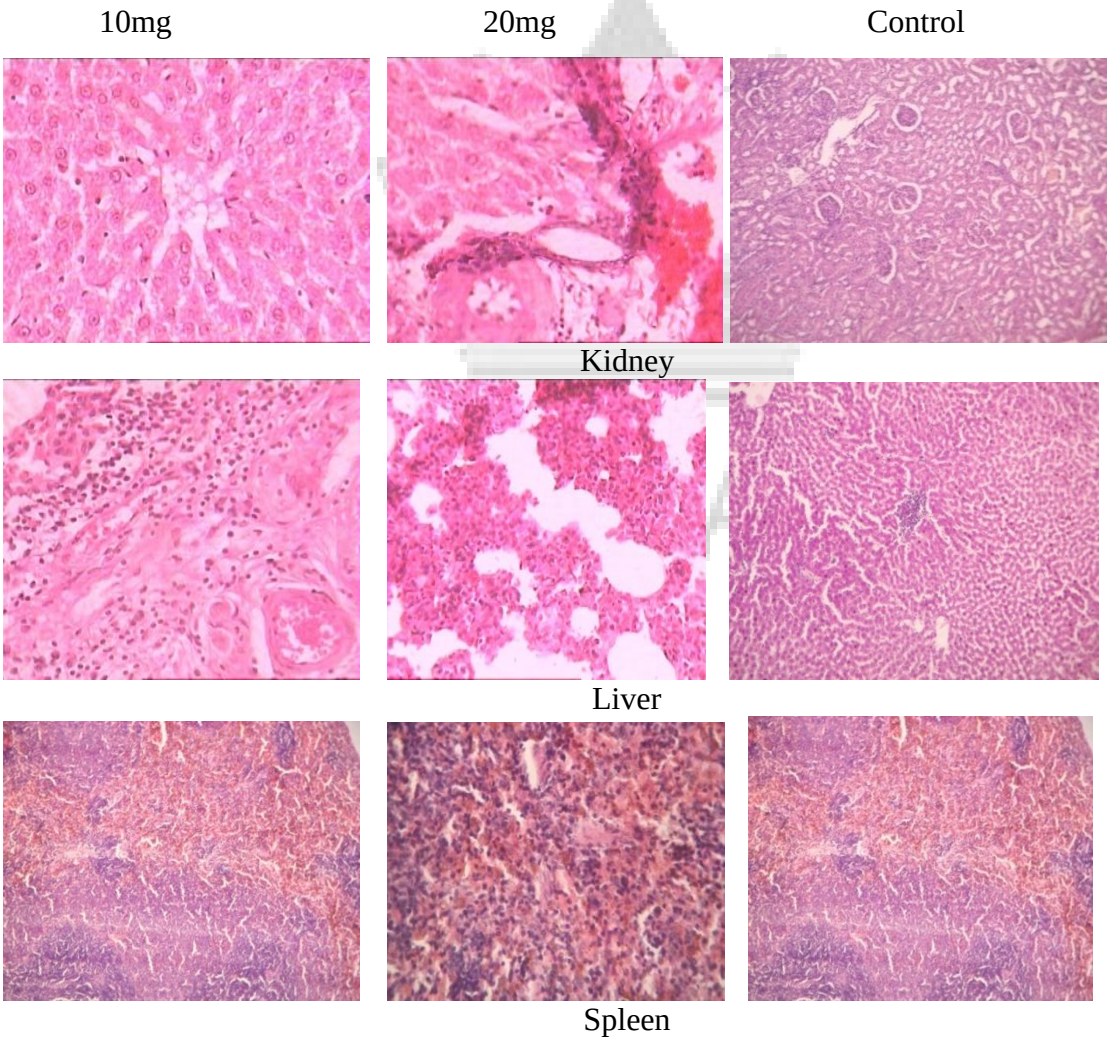

Fig. 1: Histopathological slides

DISCUSSION

The acute and repeated 28 days oral toxicity studies of PMC showed did not produce any toxicity signs in Wistar albino rats. Daily administration of PMC at different doses 10mg/kg, 20mg/kg for 28 days were tolerated by the rats without any mortality and morbidity, indicates the drug tolerance. Toxicity signs such as piloerection, salivation, tremors, signs of convulsion, lacrimation were not observed.

Mice treated with PMC at high dose 20mg showed minimal increase in Cholesterol, Triglyceride, Total Bilirubin, SGPT (Table 3). Whereas other Biochemical and haematological parameters are almost similar in all experimental groups. The histology assessment in the spleen and liver did not reveal any vascular changes. Analysis of kidney tissue revealed normal histopathology in all treatment groups.

CONCLUSION

Based on above findings, no toxic effects were observed upto 20mg/kg of body weight of PMC treated via oral route over a period of 28 days. So this study concluded that the PMC is suitable for therapeutic use in human with the dosage recommendations of upto 20mg/kg of body weight. And PMC is safe and is considered to be used widely in the clinical application.

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