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ACUTE AND SUB ACUTE TOXICITY STUDY ON SIDDHA DRUG RASA CHENDOORAM

M. Jayabharathi *[1], M. Mohamed Mustafa [1] and P. Sathiyarajeswaran [2]

Department of Sirappu Maruthuvam, Govt. Siddha Medical College [1], Chennai, Tamil Nadu, India.

Research officer [2], SCRI, Chennai, Tamil Nadu, India.

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Correspondence to Author:
M. Jayabharathi

Post.Graduate Scholar
Sirappu Maruthuvam Dept.
GSMC, Chennai. Tamil Nadu.India.

E-mail: bharathivelan84@gmail.com

ABSTRACT: Herbal and herbo mineral preparations are being traditionally used in Indian system of Medicine especially in Siddha Medicine. Herbo mineral preparations have longer Shelf life. Rasa Chendooram (RC) is prepared as per classical text book of Prana Rakshamirtha Sindhu, for Vadha diseases. Before conducting clinical trial, Pre clinical study should be undergone as per WHO guidelines .The clinical trial has been approved by IEC (IEC NO-GSMC-CH/1/2013/011).The present preclinical study aims to carry out safety and toxicity of RC (IAEC NO.XXXXIX/07/CLBMCP/2013dated 29.06.2013) Adult both sex of Swiss albino rats weighing 220-250 gm were used. Acute & Sub acute toxicity were carried out as per OECD guidelines 423 and 407. Hematological Parameters, biochemical parameters histopathological study were performed for all animals. The study concludes that on oral administration of 100mg/kg of bodyweight of RC to Swiss albino rats, there was no change in behaviour movements and no characteristic clinical sign of toxicity or mortality observed.

INTRODUCTION: Cervical spondylosis is the degenerative disease of inter vertebral discs and secondary osteo arthrosis is often asymptomatic but may be associated with neurological dysfunction, C5,C6,C7, nerve roots are most commonly affected[1]. In males, the prevalence was 13% in the third decade, increasing to nearly 100 % by age 70 years. In females the prevalence ranged from 5% in the fourth decade to 96% in women older than 70 years. 60% of population older than 45 years and 8% older than 65years of age account for the case of cervical spondylosis reported [2]. It is more commonly reported in 25 years of age now days.

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The reasons underlying are prolonged computer usage, Two-wheeler driving, poor sitting postures, and posterior neck injury [3]. Even though there are treatments available in the contemporary medical system there is no complete relief. In Siddha system for cervical spondylosis, internal medicine, external therapies and exercises are advocated by Siddhars. As per Yugi Chinthamani, Cervical spondylosis correlated with features of Ceganavadham, which is one among 80 types of Vadha disease [4].

Rasa chendooram (RC), a herbo mineral drug indicated for vadha diseases is quoted in Prana Rakshamirtha Sindhu[5]. RC ingredients are Rasam, Gandhagam and Karunthulasi. Rasam (Mercury) - Purifies blood, strengthen nerve plexus and prevent senility and increases life span[6]. In animals, on administration of small doses of mercury there is diminished the amount of oxidation of tissue and an increase the number of Red blood cells ,increase in

body weight and general nutrition. Gandhagam (Sulphur) is useful in skin diseases, splenomegaly, chronic fever and chronic Rheumatism[7]. Karunthulasi (*Ocimum sanctum*) has potent antioxidant anti inflammatory [8] and antistress activities [9].

The issues related to lack of scientific evidence about the efficacy and safety of herbo mineral remedies remains unresolved[10]. A preclinical toxicity study is mandatory in determining a safety dose for human trial [11]. Prior to the initiation of human trial the safety of the drug is to be proved [12]. The present pre clinical study aimed at evaluating the acute and sub acute toxicity of RC. This study provides vital information about efficacy and safety of RC.

MATERIAL AND METHODS:

Source of Drugs

Rasam (Mercury) and Gandhagam (Sulphur) were procured from Sanjeevi pharmaceuticals Pvt.Ltd. Chennai, traditional raw drugs dealer. Karunthulasi (*Ocimum sanctum*) leaves were collected from ABS Garden, Karipatti. Salem District. Materials were authenticated by Research Officer, (Pharmacognosy Department), SCRI, Chennai.

Purification of Rasam

Propotionate ratio of drug materials were used, Rasam –100gm, Brick powder –200gm, turmeric powder –200gm, Indian Acalypha juice (Acalypha indica) - 1.3 lt. Mercury was triturated with brick powder and turmeric power for one hour respectively and washed with water. Whereas the mercury boiled with the juice of Indian acalypha to get purified Rasam, detoxification procedure was carried out [6].

Purification of Gandhagam

The kalkam of *Lawsonia inermis* (100 gm) was mixed with curd of cow (1 Kg) and placed inside mud pot. The mouth of the pot was covered with a cloth. Sulphur (200gm) was placed over this cloth. The pot was covered with another pot and buried into the ground. The outer pot was subjected to puda with five dung cake. The suphur which melts and settles down at bottom was collected, and this procedure was repeated for 7 times. [6].

Preparation of Rasa chendooram

Purified Rasam and Gandhagam (each 35gm) were grind with karunthulasi juice in kalvam (stone mortar) then dried this material keep into bottle (kasi Kuppi). After this procedure, Rasa chendooram prepared by vaaluga enthiram, treat under the flame of deepakkini (mild flame-24 hour), kamalakkini (moderate flame24 hour) and kadakkini (high flame-24 hour) respectively.

Adjuvant: Amukkara chooranam (1:10 ratio)

Chemicals and Reagents & Animals

All chemicals and reagents were obtained from sigma chemicals Ltd, USA. All other reagents used in the study were of analytical grade were obtained from Qualigen fine chemicals Pvt. Ltd.

Swiss albino rats of either sex weighing 220-250grams were obtained from Animal house department, King Institute, Guindy, Chennai. Rats were housed in individually in poly propylene cages and fed with standard rodent pellet obtained and water ad libitum. The animals were subjected to a 12:12 hrs light: dark cycle under standard laboratory conditions at a temperature of 24-28ºC with a relative humidity of 60%-70%. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/XXXIX/07/CLBMCP/2013 dated 29.6.2013) of C.L Baid Metha Collelge of Pharmacy, Thurai Pakkam, Chennai, Tamil nadu.

Acute Oral Toxicity

Three female nulliparous and non-pregnant rats were used for acute oral toxicity study according to Organization for Economic Cooperation Development (OECD) guideline 423. [13], RC was administered orally 2000 mg/kg body weight of different groups of rats and absorbed for toxicological study. The animals were observed individually after dosing the first 30 mins, 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 defecation), 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 DOSE FINDING EXPERIMENT AND BEHAVIORAL SIGNS OF TOXICITY

Group	Day
Body weight	Normal
Assessments of posture	Normal
Sign of Convulsion,limb paralysis	Absence of sign(-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin colour	No significant colour change
Piloerection	Not observed
Raering/Urination/Defecation	Normal
Sensitivity response	Normal
ocomotion	Normal
Muscle gripness	Normal

Sub Acute Oral Toxicity

In this study, the animals were divided into three groups of each 6 animals (3 males and 3 females) and treated with low (50 mg/kg of body weight) and high dose (100 mg/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 50mg/kg and 100 mg/kg RC (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 evaluated weekly **(Table 2)**. Whereas this sub-chronic oral toxicity study was carried out according to OECD guideline 407[14, 15].

TABLE 2: FOOD INTAKE &BODY WEIGHT OF RATS TREATMENT WITH RC FOR28 DAYS

		Food (g/day/rat)	Body weight(g)
Control	MEAN	21.33	231.2
	SD	1.633	30.52
	SE	0.6667	12.46
Low Dose	MEAN	23.83	229.3
	SD	2.229	4.967
	SE	0.9098	2.028
High Dose	MEAN	21.5	224.2
	SD	1.871	2.714
	SE	0.7638	10108

Values are mean of 6 animals±S.E.M. (Dunnets test) ^{ns} p>0.05

Hematological and Blood Biochemical Analysis

On the 29th day, of the sub-chronic 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 3 and Table 4)** Biochemical parameter such as serum cholesterol, LDL, HDL, Total protein, SGOT and SGPT also recorded **(Table 5 and Table 6).**

TABLE 3: HEMATOLOGICAL PARAMETERS AFTER 28DAYS TREATMENT WITH RC IN RATS

		Total Red cells count ($\times 10^6$ µl)	Total Wight cells count ($\times 10^3$ µl)	Platelet count ($\times 10^3$ µl)	Packed Cell volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	MEAN	7.917	8.517	562.3	45.5	55.83	22.17	34
	SD	0.3545	0.2317	3.933	1.871	1.472	2.137	2.28
	SE	0.1447	0.09458	1.606	0.7638	0.6009	0.8724	0.9309
Low Dose	MEAN	8.35	9.45	570.2	46.17	56.17	22.67	44.83
	SD	0.2665	0.2881	2.639	1.329	1.722	2.338	3.545
	SE	0.1088	0.1176	1.078	0.5426	0.7032	0.9545	1.447
High Dose	MEAN	8.467	8.3	566.2	43.33	56.17	21.5	44.5
	SD	0.2503	0.1789	3.488	1.966	1.169	1.517	1.517
	SE	0.1022	0.07303	1.424	0.8028	0.4773	0.6191	0.6191

Values are mean of 6 animals±S.E.M. (Dunnets test) ^{ns} p>0.05

TABLE 4: HEMATOLOGICA PARAMETERS AFTER 28DAYS TREATMENT WITH RC IN RATS

		HB (g/dl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Moncytes (%)	Basophils (%)	Blood sugar®(mg/dl)
Control	MEAN	15.27	68.17	35.5	1.35	0.6833	0	75
	SD	0.3882	3.488	1.517	0.1871	0.1169	0	2.28
	SE	0.1585	1.424	0.6191	0.07638	0.04773	0	0.9309
Low Dose	MEAN	16.33	64.5	37.17	1.233	0.7167	0.1667	76
	SD	1.633	2.51	2.787	0.1506	0.1329	0.4082	1.414
	SE	0.6667	1.025	1.138	0.06146	0.05426	0.1667	0.5774
High Dose	MEAN	15.33	64.5	41.67	1.167	0.5333	0	76.17
	SD	1.033	1.643	2.944	0.1033	0.1366	0	1.835
	SE	0.4216	0.6708	1.202	0.04216	0.05578	0	0.7491

Values are mean of 6 animals±S.E.M.(Dunnets test) ^{ns} p>0.05

TABLE 5: BIOCHEMICAL PARAMETERS AFTER 28DAYS TREATMENT WITH RC IN RATS

		Serum Total Cholesterol (mg/dl)	Serum Triglycerides level (mg/dl)	Serum HDL Cholesterol (mg/dl)	Serum LDL Cholesterol (mg/dl)	Serum VLDL Cholesterol (mg/dl)	Serum Total Protein (g/dl)
Control	MEAN	101.2	45.5	27.83	44.33	35.67	5.367
	SD	2.317	1.049	0.7528	2.066	2.066	0.3724
	SE	0.9458	0.4282	0.3073	0.8433	0.8433	0.152
Low Dose	MEAN	103.7	48	30.73	46.5	36.17	4.533
	SD	1.211	0.8944	4.355	2.074	1.835	0.1862/0.0760
	SE	0.4944	0.3651	1.778	0.8466	0.7491	1
High Dose	MEAN	103.8	44.33	33.83	41.17	33.5	4.367/0.0816
	SD	2.229	1.366	1.722	0.9832	1.378	5/0.0333
	SE	0.9098	0.5578	0.7032	0.4014	0.5627	3

Values are mean of 6 animals±S.E.M. (Dunnets test) ^{ns} p>0.05

TABLE 6: BIOCHEMICAL PARAMETERS AFTER 28DAYS TREATMENT WITH RC IN RATS

		Serum Albumin (g/dl)	Alkaline phosphatase (U)	SGOT (AST) (IU/L)	SGPT (ALT) (IU/L)	Serum creatinine (mg/dl)	BUN (mg/dl)
Control	MEAN	2.65	255.7	239.5	62.5	0.8167	14
	SD	0.1049 0.0428	4.033	2.429	1.975	0.09832	1.414
	SE	2	1.647	0.9916	0.8062	0.04014	0.5774
Low Dose	MEAN	2.833	256.8	215.7	66.5	0.8	14.25
	SD	0.1862 0.0760	1.602	1.211	3.271	0.08944	1.084
	SE	1	0.654	0.4944	1.335	0.03651	0.4425
High Dose	MEAN	2.75	256.2	215.8	76	0.5667	14.67
	SD	0.251	1.835	1.329	1.549	0.1211	2.582
	SE	0.1025	0.7491	0.5426	0.6325	0.04944	1.054

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 groups 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. [16]

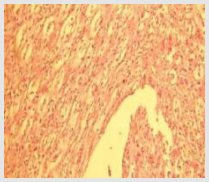
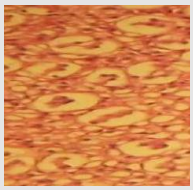
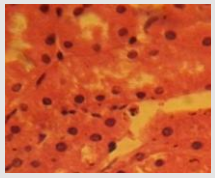
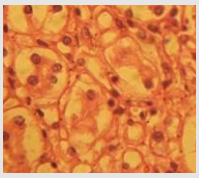
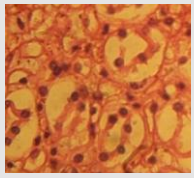
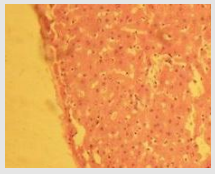
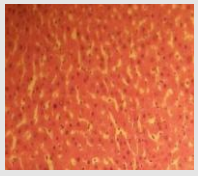
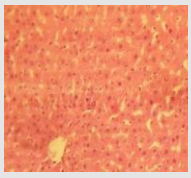
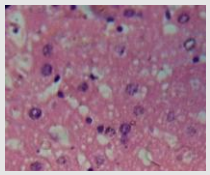
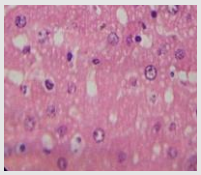
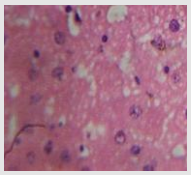
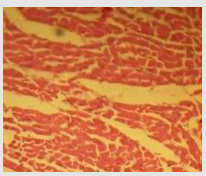
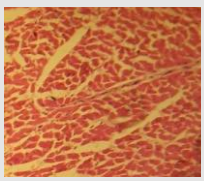
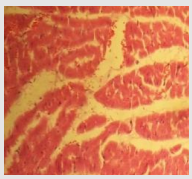
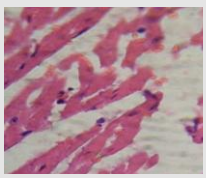
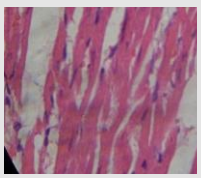
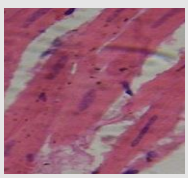
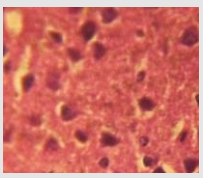
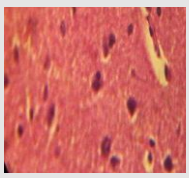
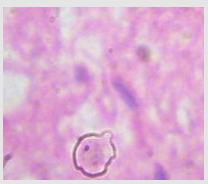
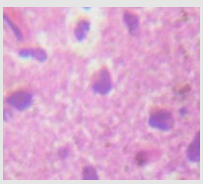
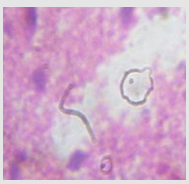
Statistical Analysis

All of the data were expressed as mean ± SEM. Statistical significance between more than two groups were using one way ANOVA followed by Bonferroni multiple comparision test. Calculations were done using GraphPad prism softwere. The significance level was set at p value ≤ 0.05 for all tests.

DISCUSSION:

- All the animals both control and dose treated groups upto 100mg/kg survived throughout the period of 28 days.
- No signs of major or significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days.
- No sign of Behavioral changes, Heamatological and Biochemical abnormalities were observed.
- Food consumption and Body weight gain were found to be comparable throughout the dosing period of 28 days.
- Opthalmoscopic examination, conducted prior to and at the end of dosing periods on animals from control and all the treated dose groups did not reveal any abnormality.
- Gross pathological examination did not reveal any abnormality.
- Histopathological examination, Liver shows Lumen of hepatic veins appears normal. No signs of necrosis. Kidney shows normal arrangement of nephrotic bundle in all the three groups Heart shows no signs of lesion or infracts was observed in all the three groups Brain shows normal histology with regular neuronal alignment further there was no considerable observation of signs of edema or degeneration.

Histopathological analysis of Sub-Acute toxicity study:

GROUP SAMPLE :	CONTROL	LOW DOSE	HIGH DOSE
KIDNEY (magnification Low power 10x)			
KIDNEY (magnification High power 45x)			
LIVER (magnification Low power 10x)			
LIVER (magnification High power 45x)			
HEART (magnification Low power 10x)			
HEART (magnification High power 45X			
BRAIN (magnification Low power 10x)			
BRAIN (magnification High power 45x)			

CONCLUSIONS: Based on above findings, no toxic effects were observed upto 100 mg/kg of body weight of Rasa Chendooram treated via oral route over a period of 28 days. So this study concluded that the Rasa Chendooram is suitable for therapeutic use in human with the dosage

recommendations of upto 100mg/kg of body weight p.o.

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