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Safety Studies of RaguRama Bana Chendhuram (RRBC), A Siddha Mineral Formulation

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***Abstract*: Ragurama bana chendhuram (RRBC) is a mercurial containing peculiar siddha formulation which is used for various diseases like soothaga vali (Dysmenorrhoea), suram (Fever), sanni (Delirium) and vayitrukazhichal (Dysentery) (1). An attempt has been made to evaluate the safety studies of RRBC in wistar rats. The acute toxicity study results show, RRBC initiation of adverse symptoms were noted from the groups treated with 500mg/kg. The mortality was observed in 1000mg/kg treated groups of animals. Sub-acute study result shows, the drug RRBC at the dose of 200mg /kg/po when administered orally for 28 days in rats did not show any toxicity in, hepatic and Haematological parameters. Further studies are to be needed to prove its therapeutic efficacy in humans through proper clinical trial.**

***Keywords*: safety studies, siddha mineral formulation**

1. Introduction

Siddha system is a known traditional medicine to human kind. It cures various ailments by using plants, metals and minerals also animal kingdom. In these sources, the minerals are having high efficacy in minimal quantity. Mercury and most of the mercurial components are used in therapeutic application for treating acute and chronic diseases [2]. Chinnabar Treats infantile convulsion, epilepsy, skin disease, sore throat and aphthae (roundish pearl-coloured specks or flakes) in the mouth and lips, etc. silver nitrate can be used for, Joint and muscle pain (rheumatism). Eye infections (conjunctivitis) and Constipation. Chendhuram is one of the 32 types of internal medicines in Siddha system of medicine. It is considered to be very potent form of drug having extensive shelf life up to 75 years and Chendhuram is oxidized product of metals or minerals, which are free from metallic residue of its parent substances. Siddha medicine describes testing of the end products by some simple checks. It insists on the color description, the drug should be fine enough to enter the crevices of finger, and it should float on the water. It ensures the correct attainment of final product. This physical testing qualifies a compound to become a drug entitled for human usage [3]. There is a need to assure the safety of herbal formulations in order to acquire their maximum benefits even though these have been proven to be efficacious in pharmacological studies or by clinical evaluation [4]. While research on medicinal plants has received considerable attention, the mineral preparations have relatively been neglected. Studies on the role of elements in health and disease have now become of global importance with rise of research activity in the last two decades [5]-[7].

2. Materials and methods

A. Ingredients

Rasachenduram (Redsulphide of mercury)-8 varagan - 32.8gms
Kadikaram (Silver nitrate) - 1 varagan - 4.1 gms
Lingam(Chinnabar) - 1 varagan - 4.1 gms.

B. Purification process [8]

Rasachenduram was soaked in the Lemon juice for 24 hrs and washed well with water. Kadikaram was soaked in Lemon juice for 12 hrs. Lingam was kept on a mud vessel and heated in low fire. The juices of Citrus lemon, Acalypha indica and cow's milk were mixed in equal proportions. The mixed liquid was poured drop by drop on lingam while heating.

C. Method of preparation [9]

The purified ingredients were powdered and ground for 2 hrs. It was stored in a vessel and cover with a suitable cork. The steamed rice was made into a paste and applied over the paper to make seelai around the mouth of the bottle. It was kept in a vessel in which paddy would be boiled. After the paddy got completely boiled the medicine was taken from the bottle and ground to make fine powder.


Rasa


Kadikaram


Chendhuram


Ragu ramabana chendhuram

D. Animal studies [10],[11]

1) Preparation of drug solution

The powdered drug was weighed in the electronic balance and mixed thoroughly and uniformly with solvent 2% Carboxy Methyl Cellulose in water to prepare the suitable stock solution. This was used throughout the study.

2) Experimental animals

Sexually mature male and female Wistar albino rats (102-134 gm) were obtained from the animal laboratory of the vel's college. All the animals were kept under standard environmental condition (27±2°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore). Male and female rats were deprived of food but not water (16-18 h) prior to administration of the RRBC. The principles of laboratory animal care were followed and the Department's animal ethical committee approved (IAEC NO: 13/IAEC/CPCSEA/VELCP/25/8.8.13) the use of the animals and the study design.

3) Acute toxicity study

Animals fasted overnight were given single oral dose of RRBC suspended in 2% Carboxy Methyl Cellulose with water.4animal groups, 6rats in each group include male and female. Age / Weight - 6 to 8wks/ 102-134 gm.

Group I, Control (2% Carboxy Methyl Cellulose 2ml p.o.)., II, III & IV treated via RRBC, 300mg/kg; p.o,500mg/kg;p.o and 1000mg/kg;p.o. Then they were observed for 14 days to record signs of toxicity and death if any. After the completion of observation period all the survivors were killed by decapitation. Necropsy was done to find gross pathological changes in the vital organs of the rats. (Table 4)

4) Sub-acute toxicity

The results of acute toxicity studies revealed that the lethal dose of RRBC was 1000 mg/kg body weight on oral drug treatment. Based on these results, the doses were selected for the sub-acute toxicity study. 4animal groups, 6rats in each group include male and female. Age / Weight - 6 to 8wks/ 102-134 gm.

Group I, Control (2% Carboxy Methyl Cellulose 2ml p.o.)., II, III & IV treated via RRBC, 50mg/kg; p.o,100mg/kg;p.o and 200mg/kg;p.o. During the period of administration, the animals were weighed, food and water intake were monitored. After 28 days of treatment all surviving animals were fasted overnight. Animals were sacrificed by decapitation and blood samples were collected via retro-orbital puncture, into heparinized tube for hematological parameters and non-heparinized centrifuge tubes. The liver, heart, kidney, stomach, brain, bone, spleen, pancreas and lung were collected and weighed. After instantaneous washing a part of the liver tissue was kept in frozen containers (-20°C) for further analysis of biochemical parameters and another part was used for histopathological studies. (Table 5).

5) Biochemical estimations

Blood collected into non-heparinized tubes were then centrifuged at 3000 rpm for 10 min. The separated serum was analyzed to evaluate the liver enzymes. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, protein and cholesterol. 20% liver homogenates were also subjected to the same biochemical estimation except that instead of cholesterol, glutathione was assayed. (Table 9)

6) Hematological assay:

Blood samples collected in the heap rinized tubes were used to investigate white blood cells (WBC), red blood cells (RBC) and platelets etc. using the visual method. (Table 8)

E. Histopathological study

Histopathological investigations of the vital organs were done. The organ pieces (3-5 µm thick) were fixed in 10% formalin for 24 h and washed in running water for 24 h. 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.

Statistical analysis: Values were expressed as mean ± SD. The statistical analyses of variance were done by ANOVA followed by the Dunnett's test.

3. Results and discussion

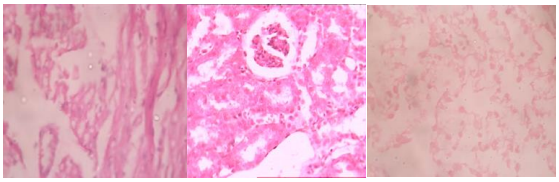
The acute toxicity study results revealed that the animals treated with 1000gm/kg dose produced remarkable changes in the general behavior pattern in the animals like slow response to external stimuli, reduction of mobility, aggression and sluggishness etc. after oral administration of RRBC initiation of adverse symptoms were noted from the groups treated with 500mg/kg. The mortality was observed in 1000mg/kg treated groups of animals. There were slight body & organ weight changes in the single dose treated animals of acute toxicity study. Therefore, the RRBC can be classified under the category of drug with moderately toxic. From the lethal dose 1000mg/kg 1/5, 1/10 and 1/20th dose were selected for further sub-acute toxic study. In sub-acute toxicity study 28 days duration was followed. During which period of drug treatment with appropriate dose levels in each group. The signs and symptoms of toxicity were noted.

The animals treated with RRBC 200mg/kg showed sudden loss of body weight after one week but later it become normal. In other dose level groups have no statistically significant body weight changes during four weeks of drug treatment. Food and water intake were progressively modified after treatment with RRBC in all the groups. (Table 5,6). ($P<0.01$; 0.05). There was minimal change in the haematological parameters. After the experimental period the biochemical results indicates that there was no significant changes in Total protein, albumin, Globulin, Chloride, HDL and VLDL. But statistically noticeable changes were observed in the levels of ALP, SGOT, SGPT, total bilurubin, Urea, Sodium, Potassium, Total cholestrol,

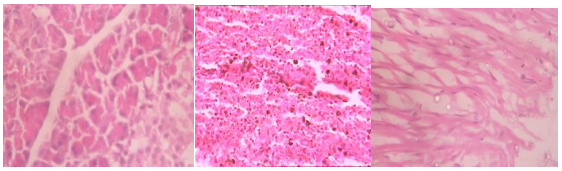
Triglycerides and Glucose. From the urine routine analysis, the drug treatment indicates changes in the P^H of urine, Protein, bilurubin, Ketones and Urobilinogen. Similarly, the vital organs weight variation confirms and alterations in biochemical parameters match with the other changes in overall parameters. So, it can be concluded that the administration of RRBC at the dose level of 200mg/kg is advisable and safe. And also, can be suggested that the dose adjustment is required based on the severity of disease in the treatment.

The histopatological study of the liver, heart, kidney, lungs, stomach, brain, spleen, pancreas, bone and testes of different groups of rats showed a normal architecture. Rats treated orally with RRBC for 28 days Liver showed little abnomalities such as steatosis clarification. The presence of steatosis also in the control groups suggested that this might be caused by diet of the animals. In conclusion, this study presents evidence of the mild toxic effect of the RRBC. These results showed that the use of the RRBC is suitable for the short period of utilization in therapy.

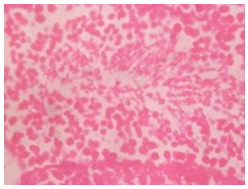
A. Histopathology Results



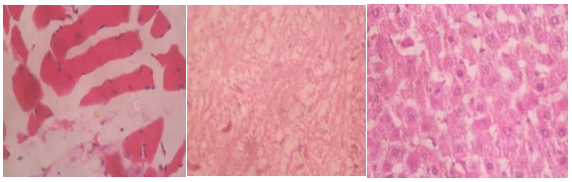
Heart Kidney Liver



Pancreas Spleen Stomach



Testes



Bone Brain Lung

Table 1
Dose finding experiment and its behavioral Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	300	+	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-
2	500	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-
3	1000	+	+	-	+	+	+	-	+	-	-	-	-	-	-	+	+	-	+	-	+

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

Table 2
Body wt (g) of rats exposed to RRBC for 4 weeks

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	115.25±4.50	118.24±5.54	121.37±5.18	126.46±7.24	130.23±8.40
50	112.88±6.43	113.32±5.16	110.60±5.80*	107.23±6.25*	105.16±6.10*
100	116.43±4.27	109.22±5.91	102.36±6.17*	103.46±6.67*	102.66±5.43**
200	113.59±4.40	98.32±4.52	100.13±7.26	100.82±7.14*	102.15±6.62**

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01.

Table 3
Food (g/day) intake of rats exposed to RRBC for 4 weeks

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	44.48±2.62	45.21±1.92	45.16±2.54	50.14±2.66	44.25±1.87
50	35.30±1.23	30.12±1.22	30.14±1.74	41.25±1.88	40.36±2.42
100	42.11±1.75	45.10±2.36	40.13±2.27	50.32±2.27*	45.12±2.19
200	45.42±2.08	40.11±1.70	40.25±1.71	46.10±2.24	40.25±2.14

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05

Table 4
Water (ml/day) intake of male and female albino rats exposed to RRBC for 4 weeks

Dose (mg/kg/day)	Days (ml/rat)				
	1	7	14	21	28
Control	60.12±1.47	60.25±1.70	55.10±2.88	60.36±2.78	44.80±2.26
50	56.64±2.28	52.12±1.78	62.15±2.80	71.10±3.00*	60.12±2.16
100	52.60±2.81	68.25±3.22*	54.20±1.82	60.32±2.34	44.48±2.60*
200	34.43±2.00	42.34±1.26*	50.11±1.00**	60.25±3.00**	52.10±4.88**

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=6

Table 5
Hematological parameters after 4 weeks treatment with the RRBC

Parameter	Control	50mg/kg	100 mg/kg	200 mg/kg
Red blood cell (mm3)	7.27±0.18	7.32±0.22	8.52±0.22*	8.73±0.23*
HB (%)	15.20±0.32	17.35±0.41*	17.10±0.57*	17.12±0.50*
Leukocyte (x106/mL)	12292±100.92	11468±130.12	13208±142.28	12870±206.15
Platelets/ul	1331±86.62	1190±55.54	1202±102.31	1218±44.82
MCV (gl)	60.12±2.44	54.94±2.45	53.92±3.00	52.34±3.02
DLC (%)				
N	4.32±0.72	10.11±1.70**	8.12±0.71**	11.34±2.40**
L	90.12±3.62	84.12±3.47	88.10±3.12	85.23±3.28
M	2.12±0.68	2.10±0.44	2.38±1.10	1.10±0.20
E	1.10±0.22	1.12±0.60	2.31±0.52*	1.18±0.31
B	0	0	0	0
ESR (mm)	1±00	1±00	2.22±00*	2.00±00*
PCV	48.12±1.90	45.64±2.00	45.38±1.70	44.34±2.22
MCH pg	19.32±0.41	18.62±1.27	18.17±0.22	18.30±0.45
MCHC g/dl	31.52±0.62	32.10±0.74	30.72±1.28	31.24±0.49

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01.

Table 6
Effect of treatment with RRBC biochemical parameters.- LFT

Total Bilirubin (mg/dL)	0.210±0.05	0.214±0.05	0.238±0.07**	0.288±0.07**
Bilirubin direct (mg/dL)	0.1±0.05	0.1±0.04	0.1±0.04	0.2±0.06*
Bilirubin indirect (mg/dL)	0.1±00	0.1±00	0.1±00	0.1±00
ALP (U/L)	342.10±10.00	162±7.25**	126±5.34**	128.25±5.58**
SGOT (U/L)	172.30±7.00	160.64±6.72	141.32±6.70**	252.15±7.22**
SGPT(U/L)	48.5±2.60	42.51±2.40	32.43±2.23**	88.16±4.10**
Total Protein (g/dl)	9.00±1.24	6.28±0.32*	7.35±0.41	7.32±0.50
Albumin (g/dl)	3.22±0.21	3.85±0.20	3.51±0.34	3.20±0.28
Globulin (g/dl)	5.5±0.30	3.22±0.24	3.90±0.28	3.78±0.32

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control*

Table 7
RFT

Dose (mg/kg)	Control	50mg/kg	100 mg/kg	200 mg/kg
Urea (mg/dL)	60.62±1.38	65.00±1.92*	61.26±2.00	77.47±1.45**
Creatinine (mg/dL)	0.70±0.08	6.72±0.06	0.82±0.08**	0.84±0.07**
Uric acid (mg/dL)	1.32±0.12	1.25±0.30	1.08±0.20	1.82±0.32*
Na m.mol	110.32±4.82	145.52±5.51**	146.30±4.11**	142.62±4.12**
K m.mol	48.20±3.52	17.28±1.52**	16.72±1.25**	18.42±1.58**
Cl m.mol	100.43±4.74	102.48±6.20	105.21±5.98	103.80±7.29

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control*

Table 8
Lipid Profile

Dose (mg/kg)	Control	50mg/kg	100 mg/kg	200 mg/kg
Total cholestrol (mg/dL)	42.80±2.42	54.2±2.48	60.12±3.21**	48.12±3.62
HDL (mg/dL)	12.34±1.30	15.23±1.20	16.21±1.23*	12.60±2.00
LDL (mg/dL)	24.11±1.72	34.16±4.10	36.12±3.28	28.03±3.12
VLDL (mg/dl)	20.90±2.12	15.16±1.47	18.32±1.28	17.22±1.02
Triglycerides (mg/dl)	107.46±5.84	82.43±2.66*	102.12±4.00	92.10±3.24
TC/HDL ratio (g/dl)	3.40±0.12	3.34±0.20	3.16±0.22	3.60±0.25
Blood glucose (mg/dl)	114.44±8.52	115.13±8.92	110.32±7.00	121.25±5.32

Table 9
Urine Analysis

Parameters	Control	50mg/kg	100 mg/kg	200 mg/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly cloudy	Slightly turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	7.2	>7.2	>8.0	>8.5
Protein	Nil	3+	3+	3+
Glucose	Nil	Nil	Nil	Trace
Bilirubin	-ve	+ve	+ve	+ve
Ketones	-ve	+ve	+ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Abnormal	Abnormal	Abnormal
Pus cells	Nil	0-cells/HPF	Nil	Nil
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	Nil	Nil	Nil
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table 10
Effect of oral administration of a RRBC on organ weight

Dose (mg/kg)	Control	50mg/kg	100 mg/kg	200 mg/kg
Liver (g)	5.22±0.18	4.32±0.12*	4.64±0.14	5.32±0.20
Heart (g)	0.62±0.04	0.48±0.05*	0.50±0.05	0.52±0.04
Lung (g)	1.60±0.06	1.00±0.05	1.14±0.05	1.10±0.04
Spleen (g)	0.62±0.05	0.32±0.03**	0.30±0.04**	0.32±0.05**
Ovary (g)	2.00±0.12	1.52±0.15*	2.73±0.10**	1.82±0.12
Testes (g)	1.32±0.10	1.30±0.08	1.27±0.12	1.48±0.28
Brain (g)	1.40±0.22	1.47±0.14	1.50±0.10	1.34±0.12
Kidney (g)	0.64±0.04	1.82±2.00**	0.68±0.08	0.62±0.05
Stomach (g)	1.42±0.08	1.00±0.05*	1.14±0.07	1.22±0.10*

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01 vs control

4. Conclusion

Based on these results, the drug RRBC at the dose of 200mg /kg/po when administered orally for 28 days in rats did not show any toxicity in, hepatic and Haematological parameters. So, it rcan be concluded that the administration of RRBC at the dose level of 200mg/kg is advisable and safe. And also, can be suggested that the dose adjustment is required based on the severity of disease in the treatment.

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