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ACUTE AND SUB ACUTE TOXICITY STUDIES OF HERBO-METALLIC FORMULATION KAALA KODI RASAM (KKR) ON WISTAR RATS

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ABSTRACT

This paper elaborates and evaluates the study of acute & sub-acute toxicity of *kaala-kodi rasam* in rat models. As indicated in Siddha literature KKR is a herbo metallic formulation treated for osteo-arthrosis. Doses of different proportions of KKR powder suspension were given to the rats for a stipulated period of time. The biochemical hepatic markers, lipid profile, renal marker changes & behaviors were noted and evaluated before and after the study. From the result obtained it is proved that the trial formulation of KKR is non toxic.

Keywords: *Kalakodi rasam*, acute toxicity, sub-acute toxicity, Wistar rats**.**

INTRODUCTION

Siddha system is the ancient system of heeling and it's based on combination of medical practices and preventive methods, as well as alchemy and mysticism. Other system of medicines is gives priority to herbal preparation for treating disease but for Siddha medicines preparation we are using combination of plants, metals, and minerals. Herbo-metallic preparations play an important role in traditional system of medicine. While such preparation are held to be safe, effective in small doses, when prepared and used following specific guidelines of Siddha text. One such effective & safe herbo metallic formulation is *kaalakodi rasam*. It constitutes *Rasam* (mercury), *Kanthagam* (sulphur), *thalagam* (arcenic). *Mercury* was a heavy metal but it is safe and effective when we purified and used in a proper procedure.

Aim and objective: To study the acute and sub acute toxicity of the trial formulation *Kaalakodi rasam***.**

2. MATERIAL AND METHODS

2.1 Collections of drugs:

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

2.2 Method of preparation of KKR:

First make *gajili* with Purified *rasam (Hydragirum)* & *ganthagam* (*sulphur*). Then fried *thalagam (Trishulphate of arsenic), lingam (Red sulphide of mercury), naabi (aconitum napellus root), nearvalam (croton tiglum seeds)), sukku (zingiber officinale root), arisithipilli (piper longum fruit), milagu (piper nigrum fruit), ,kadukai (terminalia chebula fruit) thaanrikaai (terminalia bellirica fruit),nelli (phyllanthus emblica fruit), nerinji (tribulus terres fruit)* are in a pan and make it as powder .Then mix with *Alli kilangu* juice and grind in a *kalvat* for 3 *samam* (9hrs) and shadow patterns, shrinking the size into 130mg and dryer & shut in a ceramic

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container.

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/32/CLBMCP/2014

They were kept in C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai,

2.3.1 Animal selection and maintenance:

Table: A

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	Rodent pelleted feed
	Sai Meera Foods, Bangalore)
Water	RO purified water
Identification	Rats will be kept in individual Cages and numbered
Duration of Study	48 hrs
Evaluation	14 Days

2.4 Housing and Feeding conditions

They were fed with a balanced standard pellet diet procured from SaiMeera Foods, Bangalore and maintained under standard laboratory conditions, providing 19-25^{0}C temperature, relative humidity 30-70% standard light cycle (12 h light, 12 h dark) and water Ad libitum. Unlimited supply of drinking water and conventional lab diets were adopted. Rats were kept in individual cages with raised floors of wide mesh to prevent coprophagy and numbered. Animal welfare guidelines were observed during the maintenance period and experimentation.

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:

2.6.1 Drug Stock solution:

The powdered form of *KAALA-KODI RASAM* 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.

2.6.2 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.

2.6.3 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.

2.7 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 behavior 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.

3.1. 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.

3.2. 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.

3.3 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.

4. SUB ACUTE TOXICITY STUDY OF *KAALAKODI RASAM* ON WISTAR RATS (OECD – 407 GUIDELINES) (Repeated dose 28-day oral toxicity study)

4.1. 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. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals that die or are euthanized during the test are necropsies and at the conclusion of the test surviving animals are euthanized and necropsies.

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).

4.2. 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. Each animal was fur marked with picric acid, marked to permit individual identification, and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

4.3. Justification for Dose Selection

The results of acute toxicity studies in wistar rats indicated that *KAALAKODI RASAM* was non toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. In the literature, therapeutic dosage for *KAALAKODI RASAM* 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.

4.4. Preparation and administration of Dose:

KAALAKODI RASAM at three 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 gavages, 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.

4.5. Observations:

Experimental animals were kept under observation throughout the course of study for the following parameters and were assessed as follows**,**

4.6. 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.

4.7. Clinical signs:

All animals were observed daily for clinical signs.

The time of onset, intensity and duration of these symptoms were recorded.

4.8. Mortality:

All animals were observed twice daily for mortality during entire course of study.

4.9. 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.

4.10. Laboratory Investigations:

The laboratory investigations were carried out on day 29 in the animal's blood samples which were kept in fasting overnight. 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.

4.11. 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).

4.12. 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.

4.13. 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;

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

4.14. 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 h and washed in running water for 24 h. Samples were dehydrated using an auto technique method 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.

5. RESULTS & DISCUSSION

The result of the parameters which is observed in the sub-acute study are discussed below in the following tables

5.1 Toxicological studies- Results of Acute oral toxicity in rats

Table No.1 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
15. Lacrimation	-	Absent
16. Exophthalmos	-	Absent

The behavioral Signs **o**f the toxicity for MLM were found to be normal

Table No. 2. Body weight (g) changes of rats when exposed to Sample

Dose (mg/kg/day)	***Days***				
	0	**7**	**14**	**21**	**28**
Control	122.37±3.24	124.14±4.06	128.21±2.12	129.21±5.12	133.32±1.84
100	120.2± 3.84	122.72±6.48	123.5 ±4.4	125.43±2.64	126.8 ±6.4
200	124.5 ±3.24	130.45±5.34	132.25±3.42	134.64±3.18	137.5 ±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.

Table No. 3. Effect of sample on organ weight in rats

Organ	**Control**	**100 mg/kg**	**200 mg/kg**
Liver (g)	5.24±0.14	4.8±0.45	5.79±0.3
Spleen (g)	0.74 0.07	0.56±0.07	0.86±0.04
Kidney (g)	0.70±0.05	0.58±0.07	0.83±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 behavioral signs and mortality.

Table No. 4. Results of Hematological parameters:

Parameter	Control	100mg/kg	200mg/kg
Hemoglobin	10 ± 0.16	6.4±0.5	13.8±0.7
PCV (%)	40.2 ± 1.3	36.3±3.2	38.4±3.8
RBC(x10^{6}/mm^3)	12.6 ± 0.19	12.5±0.4*	13.2±0.16
WBC (x 10^3/mm^3)	10.12 ± 1.2	11.3±1.02	10.7±1.6
Platelet(x10^3/mm^3	900± 48.8	720± 27.8	960± 18.8
Polymorphs (%)	33± 00.14	40± 2.02	42± 00.8
Lymphocytes (%)	85± 24.8	79± 12.8	80± 38.4
Eosinophil (%)	06± 09.8	04± 0.8	05± 1.4
Monocytes (%)	0.3± 1.4	0.4± 1.2	0.6± 1.2
Basophils (%)	00	00	00

The above results showed that all hemotological parameters remained within normal limits.

Table No.5: Bio chemical parameters:

BIOCHEMISTRY	Units	Normal value	Result
Blood sugar (Random)	mg/dl	90-140	60
Serum creatinine	mg/dl	0.3 – 1.4	0.6
Serum Protein	g/dl	6.0 – 8.4	6.7
Serum albumin	g/dl	3.8 -5.0	3.6
SGPT (ALT)	IU/L	Up to 40	48
SGOT (AST)	IU/L	Up to 40	229
Blood urea (BUN)	mg/dl	08 -20	14

The above results showed that all bio chemical parameters remained within normal limits.

Table No. 6. Lipid profile:

Lipid profile	Units	Normal values	Drug treated
Cholesterol- Total	mg/dl	150 -200	106
Triglycerides	mg/dl	50 -150	71
HDL cholesterol	mg/dl	35 - 75	20
LDL cholesterol	mg/dl	80 - 100	71.8
VLDL cholesterol	mg/dl	10 - 30	14.2
Cholesterol/HDL Ratio		< 5	5.3

The above results showed that lipid content of blood remained within normal limits.

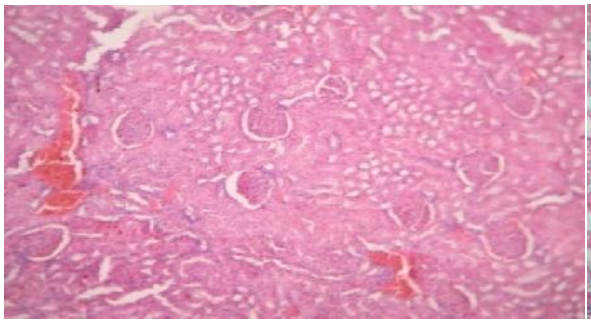
HISTOPATHOLOGY REPORT MACROSCOPIC & MICROSCOPIC EXAMINATION:

1. KIDNEY:

Received both kidney specimens measuring 1×0.6×0.5cm

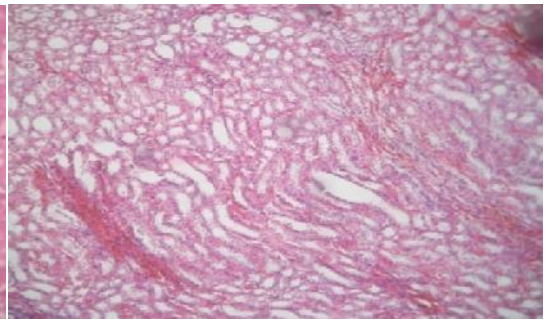
Section studied shows congestion of peril tubular vessel. The glomeruli and tubules show normal histology.

Picture 1



Control

Picture 2



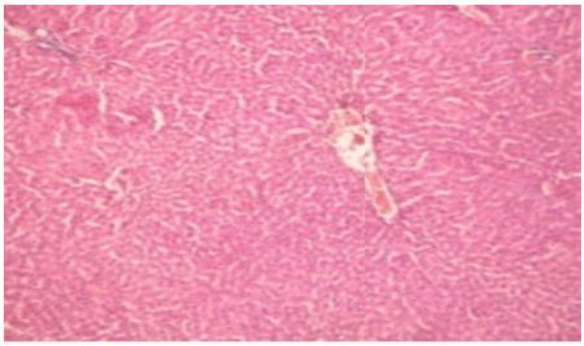
High dose

2. LIVER:

Received liver specimen mass measuring 5×4×3.5cm

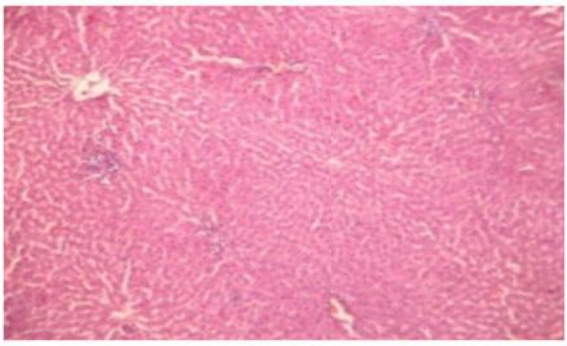
Section studied shows central venous dilation and sinusoidal dilation.

Picture 3



Control liver

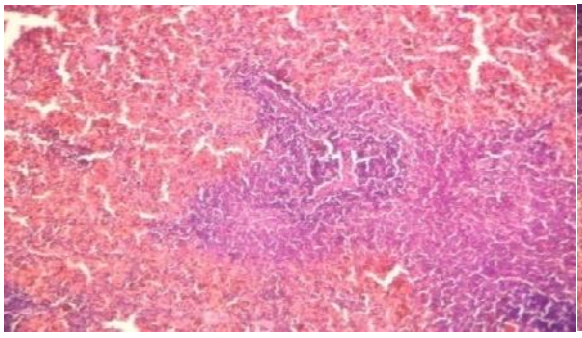
Picture 4



Drug Treated

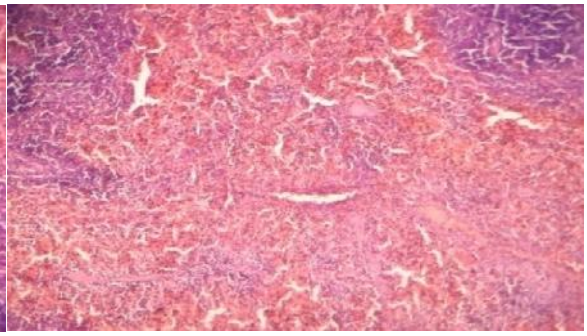
3. SPLEEN:

Picture 5



Control
(Intra parenchymal hemosiderin)

Picture 6



Drug treated SPLEENS
(Laden Macrophages)

CONCLUSION

The acute and sub acute toxicological study of *kaalakodi rasam* in rat modals is studied completely and the results obtained were found to be satisfactory. The behavioral signs of the toxicity for MLM were found to be normal. The body weight of the rats did not differ and remained within the controlled limits. The hematological, bio-chemical, lipid profile contents remained within the normal limits. The histopathological study also reveals that the kidney, liver and spleen functions were good. Thus it is evident from the studies there is no mortality among the rats and it clearly shows there is no toxicity and adverse effects of using *kaalakodi rasam.* Thus *kaalakodi rasam*

(KKR) can be used as a herbo-metalic drug for the treatment of osteo-arthrosis.

REFERENCES

1. Department of AYUSH, The Siddha pharmacopeia of India, part I, volume I, first edition, page no 158.
2. 2.prof.P.K.Ramachandra Reddy, Vaithiyarathna chinthamani, volume I, first edition, Chaukhambha Oriental publishers, Varanaasi, 2013.
3. H.B Waynforth & P.A.Flecnel, Experimental and surgical techniques in rat, second edition, 1992,page no – 24
4. Alfredo Rigalli, Veronica Di Loreto, Experimental Surgical Models in the Laboratory Rat, May-19, 2009, CRCPress, page no – 132-to 134 Pages.
5. Jonalyn Manalili, Animal Handling and Restraint, Animal welfare, published on Aug 28, 2015. Page no 102 to 106.
6. R Mythilypriya, P Shanthi, P Sachdanandam ,Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats- Journal of Health science, 2007.
7. John Wiley & Sons Ltd, Pharmacology Research & Perspectives, British Pharmacological Society and American Society for Pharmacology and Experimental Therapeutics, published by Creative Commons License, Volume 4, Issue 5,October 2016.
8. H.G.Vogel & F.J.Hock, Drug Discovery and Evaluation Pharmacological Assays, Springer's publications, second edition, pages – 569 t0 578. 2007.

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