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Research Article

Safety Profile of Kumarkalyan Rasa: Subchronic Toxicity Study in Wistar Rats

Shubhangi Pawar[1,*], Mahalaxmi Mohan[1], Uttarksha Ohol[1], Shishir Pande[2], Rajshree Kulkarni[2], Abhay Kulkarni[2]

[1]Department of Pharmacology, Mahatma Gandhi Vidyamandir's Pharmacy College, Panchavati, Nashik, Maharashtra, INDIA.
[2]Department of Rasashastra, A.S.S. Ayurved Mahavidyalaya, Nashik, Maharashtra, INDIA.

ABSTRACT

Kumarkalyan Rasa (KR), a proprietary herbometallic medicine, is used in children to treat a variety of health issues. Because it contains heavy metals, concerns have been raised about the safety of ayurvedic medicines containing heavy metals. Goal of current work was to conduct the subchronic toxicity of KR after prolonged intake. The experimental animals were divided into four groups (*n=6*). Three groups received oral KR suspension at dosages of 51.36, 205.44, and 513.6 mg/kg for 90 days. The vehicle treated group served as control. All of the animals were evaluated for evidence of mortality and toxicity. Over the dosage period, all treated and control group animals gained weight and gradually increased their feed consumption and water intake. But results revealed no significant difference in treated animals and control animals. Urine samples were collected 1 week before scarification for analysis. On day 91, after scarification, haematological and biochemical parameters for the treated and control animals were estimated. Treatment with KR had no effect on urine, haematological and biochemical parameters in comparison with control animals. The safety of KR administration is also supported by histopathological examination which has not shown any abnormal changes in any of the vital organs. Thus, *Kumarkalyan Rasa* is concluded as a safer therapeutic approach and can be used clinically.

Keywords: Ayurveda, *Kumar kalyanrasa*, Herbometallic, Subchronic oral toxicity.

Correspondence:
Dr. Shubhangi Pawar
Department of Pharmacology, Mahatma Gandhi Vidyamandir's Pharmacy College, Panchavati, Nashik, Maharashtra, INDIA.
Email: shpawar2009@gmail.com

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INTRODUCTION

The fundamental goals of Ayurveda are to maintain the health of people and to heal the sick. Ayurvedic system of treatment is based on *hetu* (etiological cause), *linga* (symptom/manifestation) and *aushadh* (medicine) all of which are based on the concept of *tridosha* (three main humours), *panchamahabhuta shodhana* as per physicochemical characteristics of substances (five fundamentals of the universe existence), *saptadhatu* (seven vital functionaries of human physiology).[1]

Rasashastra is a branch of ayurveda that focuses on the different manufacturing processes like *shodhana* (purification/potentiation), *marana* (incineration/calcination), *jarana* (polling), *murchana* (a method for transforming substances, specifically mercury, for therapeutic application), and other in-depth descriptions of metals, minerals, herbal and animal products used in ayurvedic medicine. The inherent advantages of *rasausadhies* (herbometallic medicine) such as their fast action, low dosage, tastelessness, longer shelf life, and greater palatability, have helped meet demands of both patient and pharmaceutical industry.[2] Herbometallic formulations have been used successfully in therapeutics for centuries. However, they have recently been scrutinised for their metallic contents, particularly the presence of heavy metals. As a result, toxicity studies are required to assess and establish the safety of these formulations.[3]

Rasasindoor (red sulfide of mercury), *mouktik bhasma* (incinerated pearl preparation), *suvarna bhasma* (incinerated gold preparation), *abhrak bhasma* (incinerated mica preparation), *loha bhasma* (incinerated iron preparation), *suvarnamakshik bhasma* (incinerated copper iron sulphide preparation) and *kanyasara rasa* (*Aloe vera* juice) are the components of KR.[4] It is used as an immunostimulant because it helps to develop non specific immunity, adaptogenic and *rasayana* (rejuvenation) effects that improve strength, antitussive and anti-asthmatic actions that help in respiratory problems, anti-bacterial effect that provides relief from common childhood infections, digestive stimulant and carminative effect that cures digestive problems, and haematinic effect that helps to increase haemoglobin levels inchildren.[5] *Ras sindoor* (red sulphide of mercury) relieves *phlegm* (cough) by acting as an *uttejak* (provocative) and *kaphasravak* (expectorant) agent. This medication has*vrushya, yogavahikalpa*,

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and *rasayana* action, which aids in the treatment of many paediatric pathological conditions such as *shwasa* (asthma), *kasa* (respiratory track disease), *rajyakshma* (king of all diseases), and *pratishyaya* (symptoms of common cold), among others.[5] *Swarna bhasma's* effects on *madhurarasa, laghuguna, madhuravipaka,* and *sheetavirya* provide immune-modulatory action. *Swarna bhasma* strengthens *rasa* and *rakta* (blood) *dhatu*, which makes it healthy for the brain, lungs, and intestine and increases immunity by acting on *pitta dosha. Swarna bhasma* (incinerated gold preparation) provides a therapeutic response in microbial infections and alleviates fatigue and weakness symptoms.[5] *Abhrak* (mica) which is found in KR has *deepana-pachana* (appetizer-digestive) and *rasayana* (rejuvenation) properties, which aid in the management of digestive problems in children, the relief of constipation, the treatment of gastric problems and the maintenance of nutritional supply in children.[5]

Despite being useful, the toxicity profile of KR has never been scientifically evaluated, so the present work was undertaken. The study was conducted as per OECD guideline 408.

MATERIALS AND METHODS

Experimentation

In this study, male and female Wistar rats weighing 150-200 g were procured from the National Institute of Biosciences, Pune, Maharashtra, India. The animals were maintained at standard conditions (22±2°C, 40-60% humidity and a 12-hr light/dark cycle) and fed ad libitum with rodent diet and distilled water. The experimental protocol was approved by the Institutional Animal Ethics Committee (MGV/PC/CPCSEA/XXXIX/01/2022-23/04). The subchronic toxicity test was conducted according to guideline No. 408 for testing chemicals of OECD.

Test drug

Kumarkalyan Rasa was acquired from Aushadhi Bhavan, Ayurved Seva Sangh, Ganeshwadi, Panchavati, Nashik, Maharashtra, India. It was shielded from light and moisture while being stored at ambient temperature (25 to 27°C).

Preparation of test drug

The test component was water-insoluble by nature. The suspension was made using distilled water right before administration. Over the duration of the trial, the dosing volume of 1 mL/100 g was maintained.[6]

Dose calculation

The rationale for the dose selection was to allometrically convert three dose levels-low, moderate (6 times to low dose), and high (10 times to low dose) in rats using a standard conversion factor that took into account human body weight. The therapeutic dose for human weighing 60 kg is 500 mg/day. Animal dose was determined as:

Human Equivalent Dose (HED in mg/kg)=Animal dose (mg/kg)×Animal *Km*÷Human *Km*.[7]

Study design

The subchronic toxicity test was conducted according to guideline No. 408 for testing chemicals suggested by OECD.[6] A total of 24 animals were used in this investigation for the treatment groups and control group. Animals were grouped into four groups (n=6, 3 male+3 female).[8] Control animals (Group IV) administered with distilled water 10 mL/kg, p.o. Group I, group II and group III received KR 51.36 mg/kg p.o., KR 205.44 mg/kg p.o. and KR 516.3 mg/kg, p.o. respectively for 90 days using oral gavage needles (18 gauge).

Observations

General clinical observations and mortality

Over the course of the trial, animals were checked twice a day for mortality and morbidity and once a day for clinical symptoms of toxicity. Clinical indicators such as posture and convulsions observed in a home cage, handling indicators such as ease of cage removal and handling responsiveness, skin inspection, lacrimation and salivation and open field indicators (arousal, respiration, clonic movement, gait, rears, urination, defecation, mobility, stereotype and bizarre behaviour) were observed.[6,9]

Bodyweight

The animals' body weight was measured on the first day of dosing, thereafter weekly and at the end of the trial.

Feed Consumption

Daily records of each animal's feed consumption were kept.

Waterintake

Daily water consumption was monitored.

Blood collection

For haematological and biochemical analyses, approximately 4 mL of blood were drawn from each animal of every groups. Prior to collecting blood, the animals were overnight fasted. Ketamine (60 mg/kg) and xylazine (16 mg/kg)[10] were injected intraperitoneally to anaesthetize the animals. Using a disposable syringe blood was drawn from anaesthetized animals by cardiac puncture.[11,12] Blood was drawn into tubes containing EDTA for haematological analysis and tubes free of anticoagulant for biochemical examination. Blood was centrifuged using REMI centrifuge at 3000 rpm for 15 min within 1 hr of collection in order to assess the biochemical parameters of the serum.

Haematological studies

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin

Concentration (MCHC), differential WBC count, total White Blood Cells (WBC), Red Blood cells (RBC), Haemoglobin (HGB), Platelet (PLT), were analysed using haematology analyzer (Erba H 360).[13-15]

Biochemical studies

An evaluation of the Blood Urea Nitrogen (BUN), Total Cholesterol (TC), Total Protein (TP), albumin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), creatinine, Triglyceride (TG), glucose, total bilirubin, HDL, LDL and blood urea were performed using ERBA CHEM-7 biochemical analyzer.[15-17]

Relative organ weight

Brain, heart, liver, lungs, pancreas and kidneys were removed from the animal after euthanasia and the relative organ weight was determined as follows:

$$\text{Relative organ weight} = \text{organweight} \div \text{bodyweight} \times 100.$$[18]

Urinalysis

Urine was estimated during the final week of the treatment schedule for the animals in all groups. The pH by using reagent strips for urinalysis, colour, appearance, protein, glucose and blood/ blood cells of urine samples were analyzed using simple microscope with high power 45X lense.

Histology study

On day 91, all the animals were subjected for histological analysis after exsanguination. Organs were removed and bathed in formalin (10%). The tissue samples (heart, brain, liver, lungs, kidney and pancreas) were sent to K.B.H. Dental College, Panchavati, Nashik for histological study. Paraffin-embedded tissue samples were used to create Haematoxylin and Eosin (H&E) stained sections. Following staining, a light microscope (Coslab) was used to view the glass slides under 10X.

Statistical analysis

GraphPad Prism version 9.5.1 was used to conduct a statistical analysis of the data. Dunnett's Multiple Comparison test was performed after one-way ANOVA to analyse the data. The results were all displayed as Mean±SEM, $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

General Clinical Observation and Mortality

Animals in the treatment and control group showed no clinical signs of toxicity or mortality.

Body Weight

In the control and all treated groups, animals indicated a progressive elevation in body weight throughout the course of the study. In comparison to the control group, the weekly mean body weights of all the treated animals were found to be statistically non-significant (Figure 1). The proportional weight gain indicated that the KR did not have any major serious repercussions.

Feed Consumption

Animals of control and treated group showed a progressive increase in feed consumption during the course of the trial. The weekly mean feed consumption of all the treated animals was discovered to be statistically non-significant when compared to the control group (Figure 2).

Water Intake

Over the course of the study, water intake increased gradually in all of the control and treatment groups in animals. When compared to the control group, it was shown that the weekly

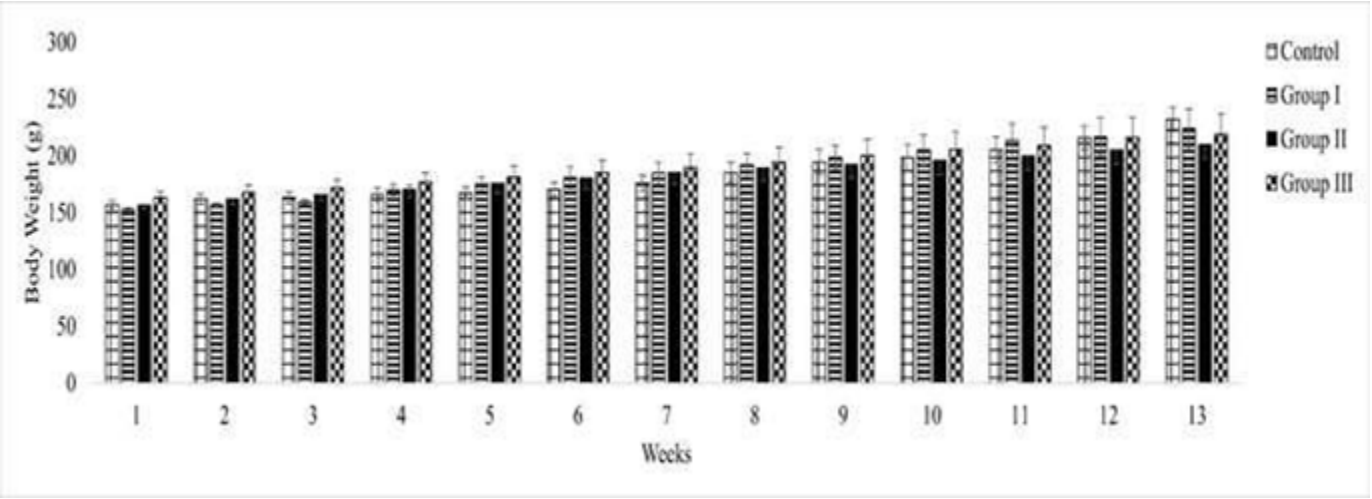

Figure 1: Effect on body weight of Wistar rats orally administered with KR for 90 days. Data expressed as mean±SE (n=6). The statistical analysis was performed using a oneway ANOVA, followed by multiple comparison with Dunnett's t test.

mean of water intake across all treated animals was statistically non-significant (Figure 3).

Haematological studies

None of the treated groups have shown any significant haematological differences from the control group (Table 1). In connection with previously done toxicity studies of herbometallic formulations there was significant increase in PLT.[18] Also, one of the prior studies done based on herbomineral formulation indicates a marginal decrease in HBG level.[19] Butin the present study there were no such findings so the KR should be considered as non-toxic by haematological approach as well.

Biochemical Studies

There are no discernible biochemical differences between any of the treated groups and the control group (Table 2). No any biochemical parameter was significantly changed by test treatment compared to control. In a publically reported research article on the safety investigation of herbomineral formulation, a considerable rise in blood sugar and cholesterol levels in male rats were observed.[19] The current study no such alterations in any of the biochemical parameters were found; hence it was concluded that KR is not toxic in almost any biochemical manner.

Relative organ weight

Animals from all groups had no significant change in the relative weight of their organs (Table 3).

Urinalysis

On day 83, when urine parameters were examined, there was no difference between the treatment groups and the control group in terms of appearance, pH, glucose, protein and blood cells in the urine (Table 4).

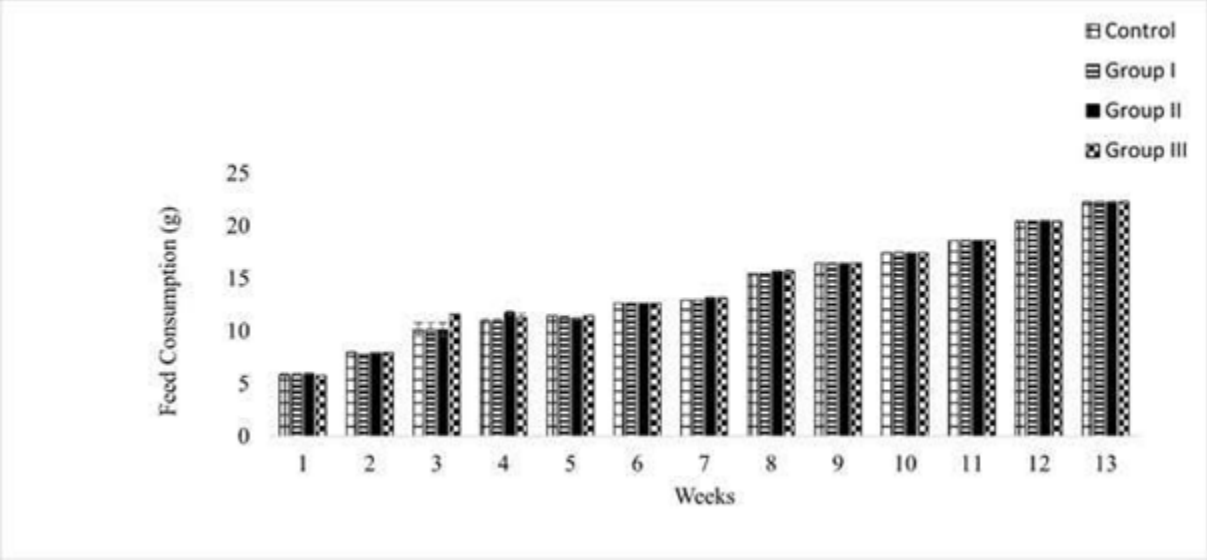

Figure 2: Effect on feed consumption of Wistar rats orally administered with KR for 90 days. Data expressed as mean±SE (*n*=6). The statistical analysis was performed using a one-way ANOVA, followed by multiple comparison with Dunnett's *t* test.

Table 1: Haematological values of Wistar rats orally treated with KRin sub chronic toxicity study.

Parameters	Control	Group I	Group II	Group III
WBC (10^3/µL)	3.72±0.49	2.60±0.39	4.74±0.55	7.06±1.10
Lym (%)	88.5±2.00	88.85±0.25	78.86±5.46	84.46±2.20
Gran (%)	2.3±0.20	3.75±0.05	4.35±0.05	4.18±0.57
RBC (10^6/µL)	8.28±0.25	7.23±0.04	7.78±0.34	8.92±0.34
PLT (10^3/µL)	667±35.00	773±26.00	851±114.30	666±67.56
MCV (fL)	52.63±2.21	52.65±1.45	50.34±0.54	52.08±0.75
MCH (pg)	36.8±0.85	37.65 ±0.45	38.24±0.54	37.1±0.73
MCHC (g/dL)	19.37±0.41	19.8±0.80	19.24±0.42	19.32±0.38
HBG (g/dL)	16±0.68	14.35±0.65	14.96±0.60	17.2±0.54

Data expressed as Mean ±SE (*n*=6).

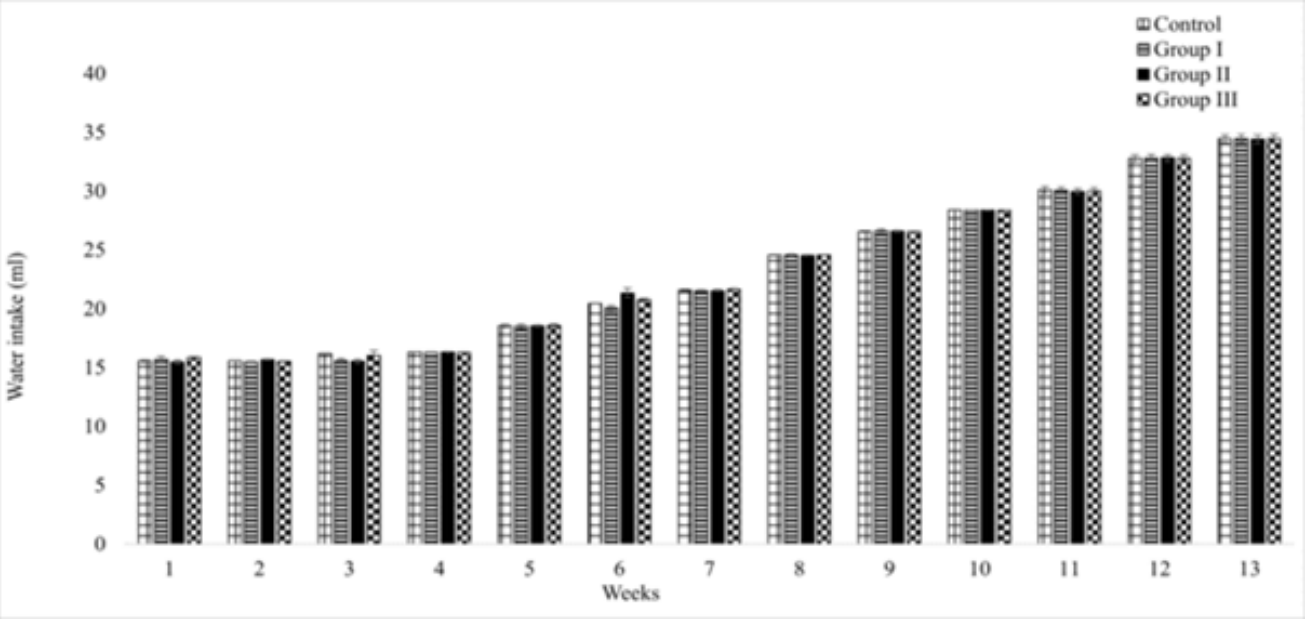

Figure 3: Effect on water intake of Wistar rats orally administered with KR for 90 days. Data expressed as mean±SE(*n*=6). The statistical analysis was performed using a one-way ANOVA, followed by multiple comparison with Dunnett's *t* test.

Table 2: Biochemical values of Wistar rats orally treated with KR in sub chronic toxicity study.

Parameters	Control	Group I	Group II	Group III
Glucose (mg%)	140±16.51	193.6±25.85	126.6±20.57	138.7±21.36
Total bilirubin (mg%)	114.5±0.10	79±0.13	93±0.09	83.67±0.13
AST(IU)	37.33±3.39	30.5±1.5	24.5±0.5	26.67±2.73
ALT (IU)	114.5±9.67	79±10.0	93±2.65	83.67±8.37
TP (g%)	6.25±0.33	6.15±0.53	6.22±0.43	6.32±0.22
Albumin (g%)	3.43±0.27	3.55±0.30	3.7±0.24	3.18±0.15
Globulin (g%)	2.63±0.41	2.96±0.45	2.96±0.20	3.13±0.28
TG (mg/dL)	86.33±11.97	74.2±7.92	104.8±6.13	78.17±5.71
TC (mg%)	102.4±16.97	133.5±18.53	115.8±17.13	104±6.21
HDL (mg%)	59±7.10	71.33±9.09	58.5±5.74	59.17±8.00
LDL (mg%)	25.67±3.48	13±0.58	21.5±2.40	35.33±5.18
Blood urea (mg%)	22.67±2.17	22.6±3.33	24.83±2.63	18.5±1.73
Creatinine (mg%)	1.05±0.11	1±0.07	1.2±0.08	0.85±0.06
BUN (mg%)	10.33±1.09	10.2±1.63	11.33±1.17	8.2±0.86

Data expressed as Mean ±SE (*n*=6).

Table 3: Organ weights of Wistar rats are orally treated with KR in sub chronic toxicity study.

Organs	Control	Group I	Group II	Group III
Brain	0.68±0.03	0.83±0.04	0.89±0.08	0.76±0.08
Liver	3.45±0.20	3.91±0.20	4.01±0.25	3.11± 0.13
Lungs	0.97±0.07	0.85±0.05	0.96±0.06	0.89±0.05
Heart	0.31±0.01	0.36±0.03	0.34±0.01	0.34±0.01
Kidneys	0.37±0.03	0.42±0.02	0.43±0.03	0.40±0.02
Pancreas	0.27±0.04	0.33±0.06	0.42±0.04	0.29±0.03

Data expressed as Mean ±SE (*n*=6).

Table 4: Effect on urine samples of orally treated Wistar rats.

Parameters	Control	Group I	Group II	Group III
Colour	Yellow	Yellow	Yellow	Yellow
Ph	Acidic	Acidic	Acidic	Acidic
Appearance	Clear	Clear	Clear	Clear
Albumin	Absent	Absent	Absent	Absent
Sugar	Absent	Absent	Absent	Absent
RBCs	Absent	Absent	Absent	Absent

Data represented urine parameters of animals of control group, group I, group II and group III (n=6).

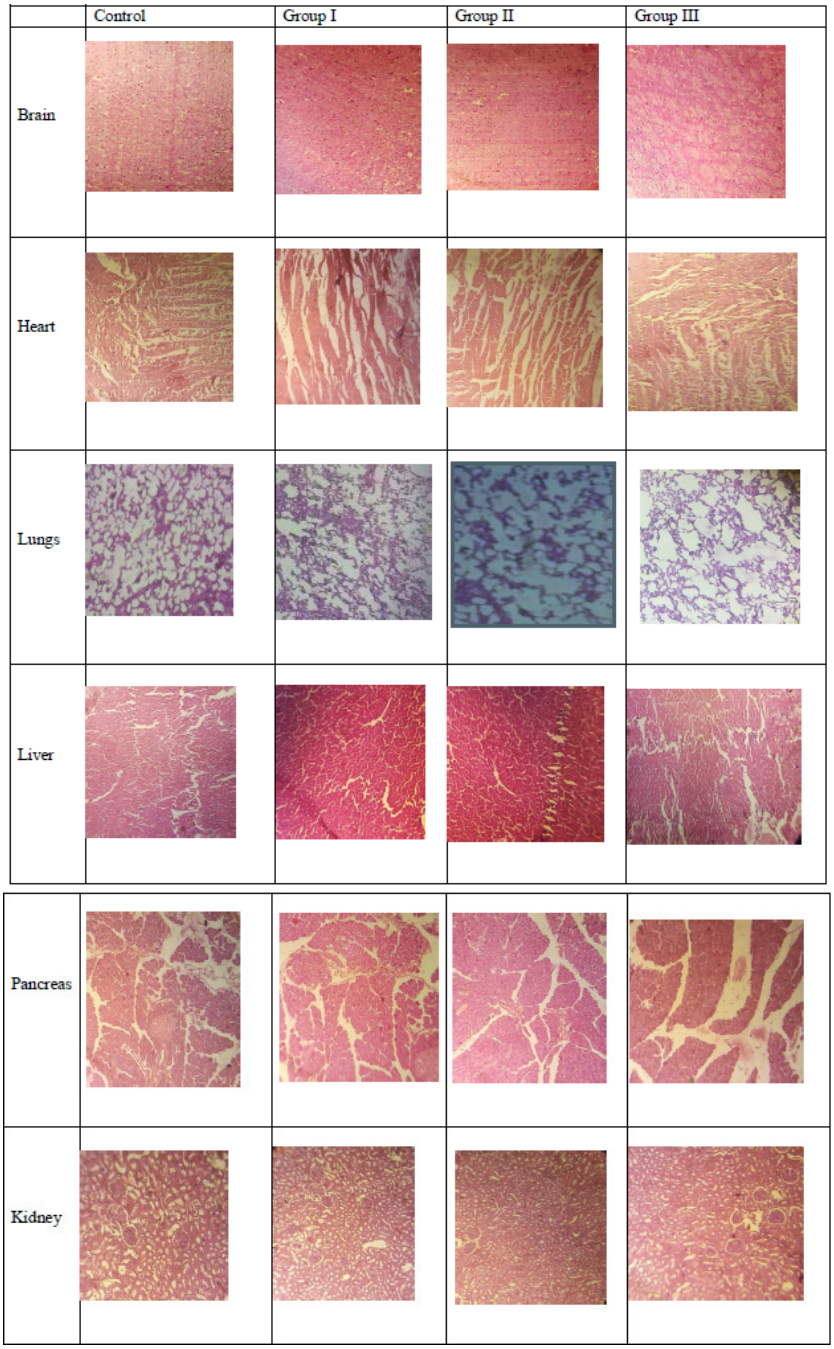

Figure 4: Photomicrographs of brain, heart, lungs, liver, pancreas and kidney taken at 10X magnification for the subchronic toxicity study in Wistar rats treated orally with distilled water, 51.36 mg/kg KR(Group I), 205.44 mg/kg KR(Group II) and 513.6 mg/kg KR (Group III).

Histopathological Evaluation

No any morphological lesions or abnormality found in any of the organs.KR therapy did not appear to be associated with any gross or histological lesions in brain, heart, liver, lungs, pancreas and kidney of animals from all groups (Figure 4).

Although presence of metal in formulation is a matter concern to use,[20] in all animals administered KR at group I (51.36 mg/kg, p.o), group II (205.44 mg/kg, p.o.), and group III (513.6 mg/kg, p.o.), there was no evidence of mortality or the moribund stage. Thus, result of the current study supports non-toxicity of KR when administered over 90 days.

CONCLUSION

The result of this study suggests that oral administration of KR for 90 days did not produce any significant toxicity in Wistar rats. Any alternation in physical, haematological, biochemical or morphological parameter was not observed. The data generated during the present study clearly showed that KR is safer for clinical use at the prescribed dose level.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

KR: Kumarkalyan Rasa; **MCV:** Mean corpuscular volume; **MCH:** Mean corpuscular haemoglobin; **MCHC:** Mean corpuscular haemoglobin concentration; **HGB:** Haemoglobin; **PLT:** Platelet; **BUN:** Blood urea nitrogen **TC:** Total cholesterol; **TP:** Total protein; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **TG:** Triglyceride.

FUNDING

This research project was funded by Aushadhi Bhavan, Ayurved Seva Sangh, Ganeshwadi, Panchavati, Nashik, Maharashtra, India.

ETHICAL STATEMENTS

The experimental protocol was approved by the Institutional Animal Ethics Committee (Proposal no.MGV/PC/CPCSEA/XXXIX/01/2022-23/04). The subchronic toxicity test was carried out in accordance with the OECD guideline 408 for testing chemicals.

SUMMARY

Kumarkalyan Rasa (KR) is a heavy metal containing herbometallic medicine so its safety is a major concern or its clinical use. The goal of this study was to determine the subchronic toxicity of KR after prolonged intake. This work was carried out as ER OECD guideline No. 408. The animals were divided into four groups (n=6). Three groups reacted with three different doses of KR for 90 days. The control group received only vehicles. All the animals were evaluated for feed and water intake, evidence of mortality and toxicity through haematological, biochemical and urin analysis parameters for the treated and control animals were estimated. Treatment with KR had no effect on urine, haematological and biochemical parameters in comparison with control animals. The safety of KR administration is also supported by histopathological examination which has not shown any abnormal changes in any of the vital organs. Thus, *Kumarkalyan Rasa* is concluded as a safer therapeutic approach and can be used clinically.

STATEMENT OF CONTRIBUTION OF RESEARCHERS

Concept-S.P.,A.K.,R.K.; Design-M.M.,S.P.,U.O.; Supervision-S.P., M.M.-; Resources-S.P.,A.K.,R.K.; Materials-U.O.; Data Collection and/or Processing-S.P., U.O.,R.B.; Analysis and/or Interpretation-U.O.; Literature Search-U.O.; Writing-M.M.,S.P.,U.O.; Critical Reviews-M.M.

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