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Research Article

Acute And Chronic Toxicity Study of Herbo-Mineral Preparation Kampavatari Rasa In Swiss Albino Mice On Oral Administration As Per Organization For Economic Cooperation And Development (OECD) Guidelines.

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Abstract

Objective: Kampavatari rasa is a herbo-mineral concoction that has been used in Indian medicine since ancient times to treat Kampavata, a condition similar to Parkinson's disease and there are currently no toxicity data available.

Methods: Acute toxicity research was conducted with 5 Swiss albino mice weighing 25 - 30 gm according to Organization for Economic Cooperation and Development (OECD) guidelines. All of the animals were given the test drug orally at a dosage of 2000 mg/kg body weight and watched for the next 13 days and they were examined for fur, eyes, nose, abdomen, external genitalia, secretions, and autonomic nervous system activity at 14th day to access for acute toxicity studies. The daily administration of test medication at the highest tolerable dose (400 mg/kg) was maintained for the following three months and studied in a manner identical to the acute toxicity study before being sacrificed and exposed to a complete blood count, liver and renal function test.

Results: Acute toxicity findings revealed no change in any of the five animals in terms of observation and response to handling, as well as no significant difference in body weight or death when compared to the control. This study reveals that Kampavatari rasa was determined to be safe at 2000 mg/kg bd. wt and any of its 1/5th (400 mg/kg), 1/10th (200 mg/kg), and 1/20th (100 mg/kg), by acute toxicity testing, and also safe by chronic toxicity following three months observation.

Keywords: Acute toxicity study, Chronic toxicity study, Herbo-mineral preparation, Kampavatari rasa

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Introduction

As per Indian system of medicine, Kampavatari rasa (Katuki swarasa, *Picorrhiza kurroa*) has been a well-known Ayurvedic herbo-mineral preparation for treating Kampa vata which is comparable to Parkinsonism as per modern pathology. The two main goals of Ayurvedic therapy are to maintain neuro-nutrition and balance the Vata dosha (nervous system disorder). Kamapavatari Rasa's contains Tamra Bhasma (incinerated copper) and Rasa Sindura (processed mercury and sulphur, which are triturated for 21 times with Katuki swaras (*Picrohiza kurroa*). Every component is integrated according to the principles of ancient Ayurvedic books (Patil Rohan & etal 2016, Mayank Kumar Malik, Pankaj Bhatt etal 2022)

The aim of the study was to evaluate acute and chronic toxicity study of Kampavatari Rasa in Swiss Albino Mice as per Organization of Economic Corporation and Development (OECD) criteria.

Methods

Approval was be obtained from Institutional Animal Ethics Committee (IAEC) before conducting experiment with Ref. No. AJIMS-IAEC/02/2023 dated 28-08-2023. three-month-old female Swiss albino mice weighing 25–30 gm was be obtained from the breeding stock of the animal house from the Department of Pharmacology and was used in our study. The animals were weighed and housed at 24^{o}C with a light/dark cycle of 12:12 hours with free access to food and water ad libitum and housed for a period of 10 days before the study. The study was conducted according to Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines. All the animals received the test drug at the dose of 2000 mg / kg bd. wt orally on day one and they observed for next 13 days making a total of 14 days and on 14th day, they were subjected to following observations of fur, eyes, nose, abdomen, and external genitalia, secretions and autonomic nervous system activity (e.g., lacrimation, piloerection, respiratory pattern, and response to handling). Body weight was measured on 14th day. Later, the daily drug administration of test drug at maximum tolerated dose (400 mg/kg, i.e 1/20th of 2000 mg/kg) was continued for next 3 months and observed similar to acute toxicity study and later was sacrificed. After 3 months, all the animals were tested for complete blood count, liver function test, renal function test and histopathological examination.

Statistical analysis

All the data were analysed by using ANOVA (Post hoc by Dunnett's multiple comparison tests). Observations were mean ± S.E.M and a p value of less than 0.05 was considered as significant.

Results

Table 1. Complete blood count

Blood parameters	Control	Kampavatari rasa
1. Red blood corpuscles (RBC) - (X 106/µL)	7.48±0.10	7.31±0.06*
2. Hematocrit (HCT) - (%)	0.45±0.03	0.50±0.03*
3. Hemoglobin concentration (HGB) - (g/dL)	12.63±0.18	12.74±0.18*
4. Mean corpuscular volume (MCV) - (fL)	54.24±0.82	53.65±0.60*
5. Mean corpuscular hemoglobin (MCH) -(pg)	38.69±0.37	37.95±0.61*
6. Mean corpuscular hemoglobin concentration (MCHC) -(g/dL)	26.25±5.59	31.71±0.18*
7. Red cell distribution width – co-efficient of variation (RDW-CV) - (%)	14.55±0.22	14.81±0.06*
8. Red cell distribution width – standard deviation (RDW-SD) - (fL)	39.01±0.24	39.51±0.12*
9. Platelet (PLT) - (X 109/L in blood)	1106.6±43.8	1123.2±36.2*
10. Platelet volume (PV) - (fl)	3±0.31	3.4±0.40*
11. Mean platelet volume - (fL)	9.55±0.18	9.84±0.03*
12. Platelet dimension width (PDW) - (fL)	10.81±0.03	10.77±0.08*
13. Percentage of giant platelet (PLCR) - (%)	0±0	0±0*
Mean ± S.E.M. *p>0.05-Not Significant		

Table 2. Liver function test

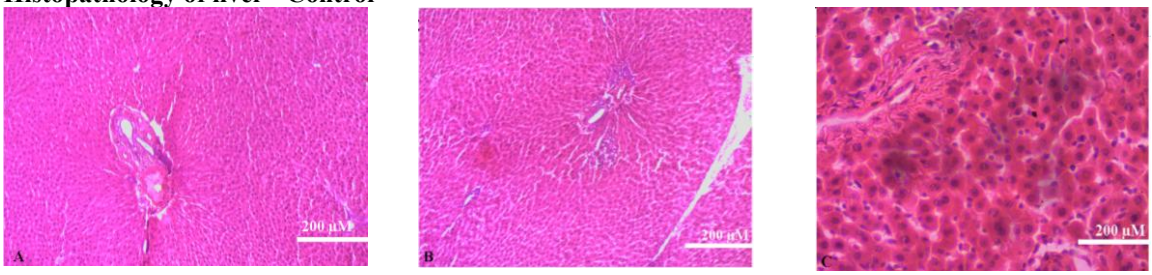
Liver parameters	Control	Kampavatari rasa
1. Alanine transaminase (ALT) - (UI/L)	58.72±0.35	59.11±0.05*
2. Aspartate transaminase (ALP) - (UI/L)	75.01±2.89	75.38±2.60*
3. Alkaline phosphatase (AP) - (UI/L)	45.33±0.45	45.32±0.72*
4. Cholesterol (CHL) - (mg/dL)	91.91±0.04	91.31±0.37*

5.	**Albumin (ALB) - (g/dL)**	1.88±0.04	1.85±0.04*
6.	**Globulin (GLB) - (g/dL)**	2.43±0.10	2.40±0.12*
7.	**Glucose (GLC) - (mg/dL)**	85.36±0.51	88.45±1.30*
8.	**Triglycerides (TG) - (mg/dL)**	95.38±0.19	95.36±0.20*
9.	**Total protein (TP) - (g/dL)**	5.98±0.20	5.55±0.20*
10.	**Uric acid (UA) - (mg/dL)**	1.40±0.01	1.41±0.14*
Mean ± S.E.M. *p>0.05-Not Significant			

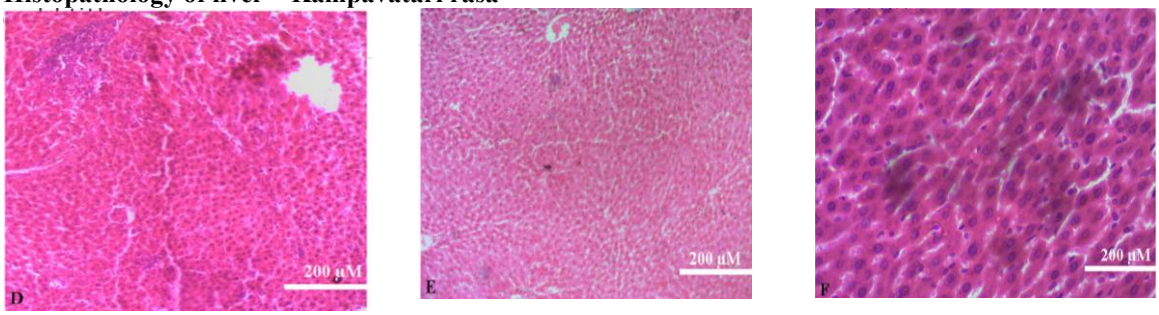
Table 2. Renal function test

Renal parameters		**Control**	**Kampavatari rasa**
1.	**Serum creatinine - (mg/dl)**	1.79±0.02	1.64±0.03*
2.	**Urea / BUN (mmol/L)**	25.46±0.26	25.28±0.19*
Mean ± S.E.M. *p>0.05-Not Significant			

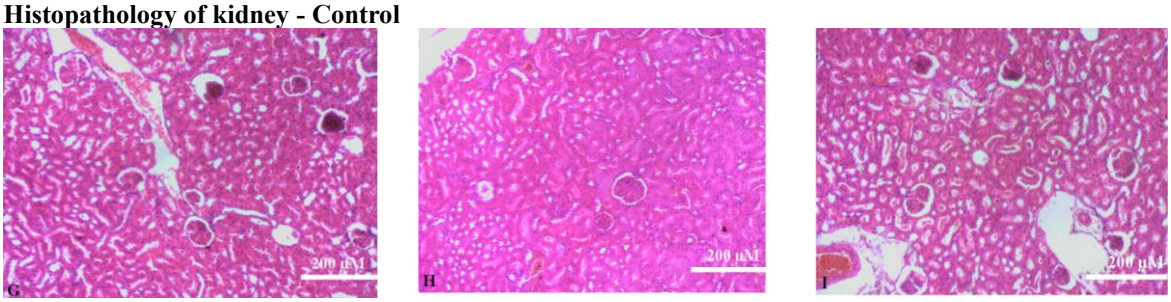
Histopathology of liver - Control



Histopathology of liver – Kampavatari rasa



Histopathology of kidney - Control



Histopathology of kidney – Kampavatari rasa

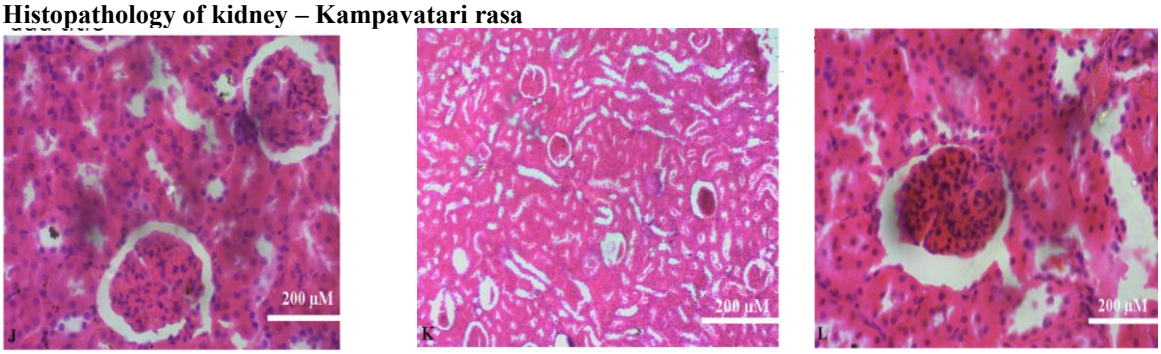


Fig. 1 Histopathology of control (1% Gum acacia 3 ml/kg) and Kampavatari rasa groups at continuous dose (400 mg/kg body weight) for 90 days.
Histology of liver (A-C) and kidney (G-I) in vehicle control and Histology of liver (D-F) and kidney (J - L) in Kampavatari rasa group

Discussion

The pharmaceutical industry and patient health rely on the safety and efficacy of therapeutic drugs Alshammari TM 2016). Herbal drugs are usually considered harmless, whereas on the contrary, the manufactured drugs are considered to be harmful to both humans and the environment (Karimi A, Majlesi etal 2015).

Kampavatari rasa's contains a proportion of copper (20.51), sulphur (8.9), and mercury, depending on its characterisation values. 3.4% which get lost during the drying process, leaving 48% total ash, 13% acid-insoluble ash, and 9.5% water-soluble ash. The test findings for tablet disintegration were 14/min and hardness were 7 kg/cm2. These results are all within normal limits and this study is the first effort to describe kampavatari rasa regarding its physical properties and ingredients. (Patil Rohan etal 2106).

Tamra bhasma is one of the most often used incinerated copper in traditional Ayurvedic medicine. It becomes toxic when the Shodhana procedure (purification) is not properly done. Tamra bhasma prepared from Shodhita Tamra is considered safe. Some data show the relevance of dosage and the role of purification that renders purified incinerated copper is safe (C. Y. Jagtap, B. K. Ashok etal 2013)

The Ayurvedic Rasagranthas (treaties) specify many doshas (impurities) for Parada (mercury), which are present in ashodit Parad (impure mercury). Shudha Parad (pure mercury) is also highlighted for its non-toxic features, capacity to ease illness, and therapeutic benefits. As a result, it becomes less toxic after a variety of Sanskar (purified procedure) and are applied to it, which is why it is used in various Ayurvedic preparations. Nevertheless, many of the applications for mercury are being examined and phased out by many modern specialists (6. (Arti, Prashant etal 2018).

Rasashastra Shodhana is a study of Samskara (process or purification technique) that eliminates impurities from a metal or mineral, resulting in modifications or adjustments in properties. All the herbo-mineral preparation including the one mentioned in this article is being used on human beings for many ailments and the toxic effects of many preparations are not yet established and Kampavatari rasa is one among that, hence the present study was under taken to access its safety.

Acute toxicity data revealed no change in observation or response to handling in any of the five animals, nor was there a significant difference in body weight when compared to the control. This study revealed that Kampavatari rasa is safe at 2000 mg/kg bd. wt, and any of its 1/5th, 1/10th, or 1/20th can be used for further pharmacological assessment.

Chronic administration of the test medication at a dosage of 400 mg/kg for 90 days produced no difference in terms of complete blood count **(Table 1),** liver function test (Table 2), or renal function test **(Table 3).** Histopathology of the liver and kidney in the Kampavatari rasa group revealed no tissue damage as compared to the control group **(Fig 1).**

The most common Ayurvedic pharmacological procedure, known as bhavana (levigation or wet grinding of single or compound powdered drugs with liquid medium, i.e., juice/decoction/solution of plant, animal, or mineral origin), is a unique procedure. In Ayurveda, Bhavana process is also known as Samskara (process), which signifies "transformation" (Samskaro hi Gunantaradhanamuchyate) of a substance's inherent attributes, culminating in the addition of new traits or qualitative improvement in its safety and efficacy. In other words, Bhavana is the incorporating the liquid media qualities into Bhavita material (drug material that has experienced the Bhavana process). Bhavana describes the properties and action (Guna-Karma) of liquid medium containing powdered medications to be levigated. Thus, it seems to alter potency (Gunantara), and introduce new attributes, or raise the potency (Guna) level of preparation (Gupta S etal 2009 Sharma R etal 2016).

Kampavatari rasa's safety profile in acute and chronic toxicity trials in Swiss albino mice was feasible owing to the processing of tamra (copper) and parada (mercury) in Katuki swarasa (juice of *Picorrhiza kurroa*) for 21 days, which would eliminate the impurities and render the product to non-toxic. However, the safety profile has yet to be demonstrated in human research, despite the fact that this medication is widely used in the Indian medical system to treat Parkinsonism.

Conclusion

The present study shows that Kampavatari rasa in found to be safe at 2000 mg/kg bd. wt and any of its $1/5^{th}$ (400 mg/kg), $1/10^{th}$

(200 mg/kg), and 1/20th (100 mg/kg), by acute toxicity study and also safe by chronic toxicity study compared to control. Hence, oral administration of Kampavatari rasa can be taken for further evaluation of therapeutic preclinical pharmacological evaluation in animal models followed by its assessment in clinical trial at later stages.

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Conflict of interest

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