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Evaluation of acute toxicity of *Rasaparpati* - An Ayurvedic mercurial compound in Albino rats

Research Article

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Abstract

Background: This study aimed to investigate the preparation process of *Rasaparpati*, an Ayurvedic medicine containing Mercury that is commonly used to treat various ailments. The dangers of using Mercury as a therapeutic agent are acknowledged, and Ayurveda emphasizes purifying heavy metals before human use. The present study assessed the acute toxicity of *Rasaparpati* in albino rats to ensure its safe consumption. Methodology: Following ethical guidelines and O.E.C.D. (423) protocol, *Rasaparpati* was prepared traditionally, and an acute toxicity study was conducted. Two groups of female Wistar rats were administered 300mg/kg and 2000mg/kg of *Rasaparpati*, and their behavioral changes, signs of toxicity, and mortality were closely monitored. Hematological, biochemical, and histopathological parameters were also examined for any changes. The data obtained were analyzed and evaluated using an unpaired t-test, with $p < 0.05$ considered significant. Results: No deaths were observed following the administration of *Rasaparpati* in an acute toxicity study. There were no hematological or biochemical toxicity indications, even at ten time the therapeutic dose. However, the histopathological analysis revealed mild changes likely to heal and repair. These non-specific and reversible changes in rat cells suggest similar outcomes in human cells. Conclusions: The acute toxicity study of *Rasaparpati* suggests its safety for animal use, indicating potential safety for human consumption. No adverse effects or mortality were observed, even at a dosage ten times higher than the therapeutic dose. However, more extensive research with larger sample sizes and control groups is recommended.

Keywords: Rasaparpati, HgS, Acute toxicity, O.E.C.D., Safety.

Introduction

For thousands of years in India, Ayurvedic and Siddha therapeutic medicines containing Mercury have been developed and utilized to treat various ailments, ranging from the common cold to cancer. (1) A comparable situation has been witnessed in other countries where traditional systems of medicine are practiced. All forms of Mercury, including vapor, inorganic salts, and organic forms, pose a hazard to human health. Extensive research has shown the harmful effects of exposure to Mercury in animal studies and in humans who have been exposed to it inadvertently. (2) Mercury has the potential to cause damage to various bodily systems, including the nervous, digestive, and immune systems, as well as the lungs, kidneys, skin, and eyes. According to the World Health Organization, Mercury is among the top 10 hazardous compounds that pose a public health concern. (3)

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Ayurveda contains numerous Mercury preparations, including *Kajjali*, *Parpati, Rasasindura, and Makaradhwaja*. (4) One such preparation, Parpati Kalpanas, is a therapeutic metallic/herbo-mineral flaky preparation utilized in Ayurveda since the 11th century A.D. for treating *Grahani* (Tropical sprue). (5) *Rasaparpati*, on the other hand, is created from purified *Parada* and purified *Gandhaka* (Sulphur). When the melted *Kajjali* is pressed between banana leaves, it produces a thin flake. This unique Ayurvedic mercurial formulation containing purified Mercury is highly potent, cost-effective, and has significant therapeutic value. While functioning as a *Rasayana*, it can treat various illnesses, including *Grahaniroga, Rajayakshma, Kustha, Gulma, Atisara, and Raktapitta*. (6)

Ayurvedic physicians have traditionally used *Rasaparpati* to treat various disorders. Despite being used for over a millennium without any reported adverse effects, objective and verifiable data is lacking to support its safety claims. According to Ayurvedic principles, the processed Mercury and Sulphur are converted to mercury sulfide, which exhibits therapeutic properties at appropriate doses without causing toxicity in humans. (7) Through unique preparation processes like *Shodhana* (purification) and *Marana* (incineration), metallic preparations undergo chemical transformations

that detoxify the raw materials and modify their properties to enhance their therapeutic potential. (8) Ongoing discussions surround the safe use of Ayurvedic medicines that contain Mercury and other metals. Conducting scientific evaluations is essential to ensure the safety of metallic drugs. Preclinical studies are vital in confirming their efficacy and safety while providing a scientific foundation for their traditional use. (9)

This study aims to evaluate the toxicity effects of *Rasaparpati* and determine its potential for acute toxicity in humans, estimate safe acute doses of toxicity for humans, and identify potential target organs of toxicity. This investigation involves assessing the preparation process of *Rasaparpati* and evaluating its acute toxicity using an experimental animal model.

Materials and Methods

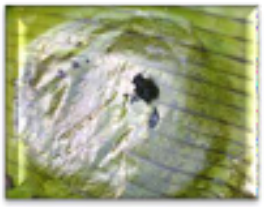
Test drug

Unprocessed *Parada* (Mercury) and *Gandhaka* (Sulphur) were obtained from the local market. These substances were then purified using classical procedures mentioned in *Rasatarangini* text of Ayurveda. The resulting *Kajjali* was obtained by rubbing equal amounts of purified Mercury and Sulphur in a *Khalwayantra* until the Mercury particles vanished, leaving a fine black powder. The *Kajjali* was heated with a gentle flame directly on the *Angarakosthi*, at a temperature ranging from 110 to 120 °C. It was then poured onto a banana leaf surface to create a thin flake of *Rasaparpati*. The analysis of both the raw drugs and finished products was conducted at CRF, KLE BMK Ayurveda Mahavidyaalaya, Belgavi, Karnataka and IIT, Mumbai as per the standards outlined in the Ayurvedic Pharmacopeia of India(10-12).

Table 1: Analytical study of *Rasaparpati*

Sl.No.	Physico-chemical parameters and analytical study	Results
1	Organoleptic Parameters of Rasaparpatis through sensory organs	When crushed between teeth, it does not produce a metallic sound; on touch, there are no coarse particles, only a smooth texture. It has a black color, a bland flavor, and an odorless scent.
2	Loss on drying at 110°C	0.414
3	Total ash value % through ash content analysis	0.487
4	Acid insoluble ash %through ash content analysis	0.194
5	Sulfated ash %through ash content analysis	0.490
6	Namburi Phase Spot Test (NPST) through principles of Chromatography	A moderately deep brown spot formed immediately, separating into a central brown spot and a brown periphery. Some white space was visible between the central spot and the periphery, marking the first phase.
7	Particle size analysis using a Carl Zeiss microscope (ZEISS Particle Analyzer)	0.757-1.337 (in microns)
8	Inductively Coupled Plasma - Atomic Emission Spectrometry (I.C.P.- A.E.S.) % Hg and S (SPECTRO Analytical Instruments GmbH, Germany)	The Hg content is 32.51%, and the S content is 64.92%. Additionally, other metals, such as Cu, are present at a percentage of 0.00065, while P.B., As, and Cd were not detected (<0.01PPM).

Figure 1: Preparation of *Rasaparapti*

A. Melting of Kajjali	B. Placing the melted Kajjali on a bed prepared with cow dung and covering it with a banana leaf	C. Pressing the Kajjali with a Pottali made with cow dung	D. Preparation of Rasaparpati	E. Final prepared Rasaparpati
				

Experimented Animals

Female Wistar rats weighing 150-200 gms, bred in the animal house of K.L.E.U, Shri B.M.K. Ayurveda Mahavidyalay Belgaum, were used for the study. The rats were maintained under standard husbandry conditions with a temperature of 22°C ± 3°C, 45-55% relative humidity, and a 12/12 hr natural light & dark cycle. They had access to a standard pellet diet and fresh water ad libitum. The Institutional Animal Ethics Committee (I.A.E.C.) of K.L.E. University's Shri B.M.K. Ayurveda Mahavidyalay, Belgaum (BMK/ IAEC/Res. No-16/2016) approved the experimental protocol following the guidelines of the Committee for

Control and Supervision on Experiments on Animals, India.

Preparation of dose

Suspensions of the test drugs were prepared using 10 ml and 5 ml of finely powdered drugs to achieve a dosage of 300mg/kg and 2000mg/kg body weight, respectively. These suspensions were mixed with a non-toxic binding agent, 10% gum acacia.

Calculation of dosage

To determine the appropriate dose of *Rasaparpati* for rats, Paget and Barner's formula based on surface area ratio was used. The resulting calculated dose was 22.4mg/kg body weight, and fresh test doses were prepared on the day of experimentation based on the animal's weight. The selected doses of 300mg/kg and 2000mg/kg were administered orally using an intubation cannula attached to a 2 ml plastic syringe. The dose volume should not exceed 1 ml/100g of body weight.

Acute Toxicity Study

Following OECD 423 (13), the acute toxicity study selected healthy, young, nulliparous, non-pregnant female albino rats of the Wistar strain. The rats were acclimatized for seven days before being divided into two groups based on the doses of the trial drug: 300 mg/kg and 2000 mg/kg. The study included a total of nine animals, three animals in Group 1 and six animals in Group 2. (Table 2)

Table 2: Animal grouping and dose selection

Group	Dosage of Rasaparpati administered	No. of animals (female)	Vol. of suspension made in 10% gum acacia for single oral administration
1	300mg/kg body weight	3	100mg in 1ml
2	2000mg/kg body weight.	6	200mg in 1ml

Three healthy rats were orally administered *Rasaparpati* in powder suspension at 300mg/kg body weight after being placed in metallic cages following an overnight fast. The rats were monitored for 14 days for signs of toxicity, mortality, and behavioral changes, with checks during the first 24 hours and daily reviews afterward. On the first day, the drug was administered. If there were no deaths, or at least one death, then on the following day (i.e., the second day), another three animals were administered a higher dose of 2000 mg/kg body weight. the same dose, which was repeated on the next day to confirm the results as there was no mortality and they were observed for 14 days. Food and water consumption, as well as body weight, were recorded weekly (Table 3). On the 15th day, blood samples were collected for biochemical and hematological analyses, and after euthanasia, morphological and histopathological changes in the Liver and kidneys of the rats were observed. (Table 3, 4, 5)(Figure 2 and Figure 3).

Table 3: Food consumption, water intake, and weight variation of animals were measured at different dosages

Study groups	Food (g) mean value	Water (ml) mean value	Weight (g) mean value (mean±SD)	
			1st day administration	14 days after administration
300mg/kg body weight dose	14.7	26.09	159.33±14.01	159.66±17.21
2000mg/kg body wt. dose	13	20.68	164.83±4.919	160.2±3.03

Figure 2: Phases of the acute toxicity investigation

A. Albino Rat placed in the metabolic cage for observation	B. Rasaparpati administered in the form of a suspension	C. Administration of the trial drug through a syringe	D. Collection of blood samples for analysis	E. Dissection of the animal for organ examination	F. Dissected animal observed for any morphological changes.
					

Statistical analysis

The data is presented as the mean ± S.D. To determine the significance between different dosages, an unpaired t-test was conducted with $p < 0.05$ considered significant.

Results

The standard protocols were followed to prepare and analyze *Rasaparpati*. In the acute toxicity study, female Wistar rats were administered a single dose of 300mg/kg and 2000mg/kg body weight of *Rasaparpati*.

A total of nine animals were used in the study. In Group 2, six animals were administered the substance, as opposed to the usual three. This increase was necessary due to the experimental protocol outlined in the OECD guideline 423. According to this guideline, if no mortality occurs after the initial dose is given to three animals, an additional three animals must be included to verify the absence of lethality. No significant toxic symptoms were observed except for mild itching in some animals. The animals' serum biochemical parameters and hemoglobin levels were within the normal range, and no animal deaths were reported (Table 4,5,6). However, histopathological analysis revealed minor toxic effects in the Liver and kidney, which were more pronounced in the higher dosage group (Table 7). The observed changes were reversible, and *Hingulottha Parada* was considered safe even at 2000mg/kg body weight. (14) In this study, we administered a dose ten times higher (300mg/kg body weight) than the therapeutic dose, which may have resulted in the observed mild damage (Figure 3, 4).

Table 4: Effect of *Rasaparpati* treated with different dosages on levels of A.L.P., serum total bilirubin, total serum protein, and serum albumin

Study Groups	ALP (In IU) **(Normal = 20- 115IU/L)**	S. BILIRUBIN (T) (In mg%)	S. TOTALPROTEIN (In gm%)	S. ALBUMIN In gm% **(Normal = 3.5- 5.0gm%)**
300mg/kg body.wt dose	64±7.21	1.06±0.20	4.2±1.48	3.7±0.1
2000mg/kg body wt. dose	75.8±5.71	0.84±0.16	6.9±0.21	3.62±0.34

Values expressed as mean ±S.D for (n1=3, n2 =6). These are significant with p-value <0.05

Table 5: Effect of *Rasaparpati* treated with different dosages on levels of S.G.O.T., S.G.P.T., urea, creatinine, and the A/G ratio

Study Groups	SGPT (In IU) (Normal = 10-40IU/L)	SGOT (In IU) (Normal = 10- 40IU/L)	S. UREA (In mg/dl) (Normal = 15- 40mg/dl)	S. CRETININE (In gm%) (Normal = 0.6- 1.2mg/dl)	A/G RATIO (Normal = 1-2)
300mg/kg body weight dose	23±2	38±1.73	26±8.88	0.6±0.1	1.06±0.05
2000mg/kg body weight dose	23.2±3.03	34.2±3.03	28.2±3.42	0.94±0.15	1.12±0.04

Values are given as mean ±S.D for (n1=3, n2 =6). These are significant with a p-value <0.05.

Table 6: Effect of *Rasaparpati* treated with different dosages on lymphocyte count, hemoglobin level, and total leukocyte count

Study Groups	Lymphocyte (in %) **(Normal = 45-75)**	Haemoglobin (in gm%) **(Normal = 12.5-14.5)**	Total Leucocytes Count (in cells/cumm) **Normal = 6000-15000**
300mg/kg body weight dose	83±0	13.93±0.90	7100±2291
2000mg/kg body weight dose	80.8±2.58	13.14±0.82	6740±1934

Values are given as mean ±S.D for (n1=3, n2 =6). These are significant with p-value <0.05.

Table 7: Histopathological changes among animals receiving different dosages

Group	Organs	Observation
300mg/kg body weight dose	Liver	Congestion ++, inflammation ++ one animal shows spotty necrosis+ and fibrosis+
	Kidney	congestion ++
2000mg/kg body weight dose	Liver	Congestion ++, cholestasis +, bile duct proliferation ++, portal triditis +, piecemeal necrosis in one animal, fibrosis in two animals
	Kidney	Congestion ++, peritubular inflammation +

Figure 3: Histopathological characteristics of Liver and kidney at a dose of 300mg/kg body weight

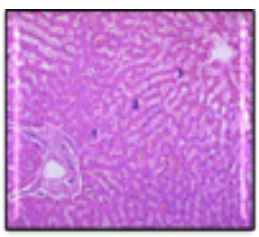
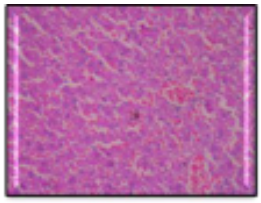
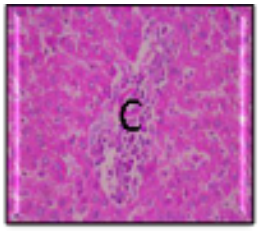
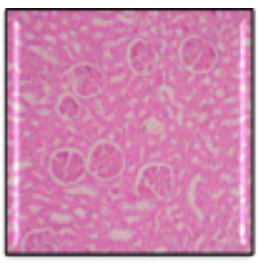
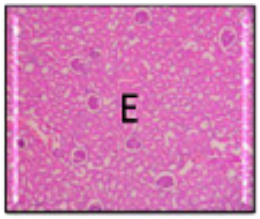
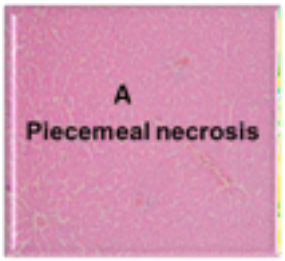
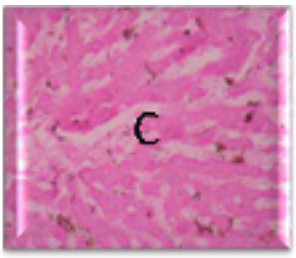
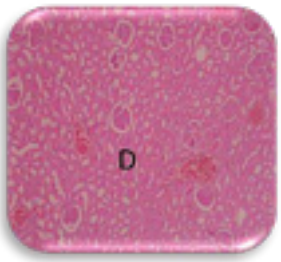
A. Liver in a normal state	B. Liver exhibiting signs of congestion	C. Liver showing sporadic necrosis	D. Kidneys in a normal state	E. Kidneys exhibiting signs of congestion
				

Figure 4: Histopathological features of the Liver and kidney at a dosage of 2000mg/kg body weight

A. Piece meal necrosis of liver	B. Fibrosis of liver	C. Cholestasis in liver	D. Congestion in kidney
			

Discussion

The presented study aimed to investigate the acute toxicity of *Rasaparpati*, an Ayurvedic medicine containing Mercury, commonly used in the treatment of various ailments. Acknowledging the potential dangers of using Mercury therapeutically, the study focused on evaluating the acute toxicity of *Rasaparpati* in albino rats to ensure its safe consumption. The study followed ethical guidelines and the OECD protocol, aiming to provide valuable insights into the safety profile of this traditional Ayurvedic formulation.

The acute toxicity study involved the administration of *Rasaparpati* at doses of 300mg/kg and 2000mg/kg to female Wistar rats. The results demonstrated a notable absence of deaths following *Rasaparpati* administration, even at the higher dosage. Furthermore, there were no significant indications of hematological or biochemical toxicity, even when administered at a dose ten times higher than the therapeutic dosage.

However, the histopathological analysis did reveal mild changes in the liver and kidney tissues. These changes, while indicative of some toxicity, were considered non-specific and reversible, suggesting the potential for healing and repair. Importantly, the study suggests that the observed histopathological changes in rat cells might translate to similar outcomes in human cells.

The outcomes of the acute toxicity study collectively indicate that *Rasaparpati* appears to have a relatively low toxicity profile in albino rats, even at doses significantly higher than the therapeutic range. This suggests a potential level of safety for human consumption. The absence of severe adverse effects and mortality, coupled with the mild and reversible nature of histopathological changes, provides initial evidence supporting the safety of *Rasaparpati*.

While the results are promising, several limitations must be acknowledged. The study's sample size was relatively small, warranting further investigation with larger sample sizes to strengthen the conclusions. Additionally, the study primarily focused on acute toxicity; therefore, conducting chronic toxicity studies could provide a more comprehensive understanding of *Rasaparpati's* long-term effects.

Furthermore, to enhance the validity of the findings, control groups and refractory groups could be included in future research. This would enable a more robust comparison of the effects of *Rasaparpati* with non-treated groups.

Conclusion

Animal testing of *Rasaparpati* for acute toxicity revealed no immediate or noticeable signs of toxicity, including hepatic and renal markers, food and water intake, body weight, and stool. No animal deaths occurred during the study. However, the histopathological analysis showed mild to moderate changes in the Liver and insignificant changes in the kidney. The study concluded that there was mild toxicity at a dose ten times higher than the therapeutic dose, with the potential for healing and repair. The observed changes were non-specific and reversible, with similarities to human cells. Further studies with a control group, a larger sample size, and an additional refractory group are recommended to investigate this further.

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