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Acute Oral Toxicity Study of Loha Parpati In Wistar Rats by Acute Toxic Class Method

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Abstract: *The present study, titled "Acute Toxicity Study of Loha Parpati in Wistar Rats by Acute Toxic Class Method", was conducted to evaluate the safety profile of Loha Parpati, a classical Ayurvedic Rasaushadhi. The objective was to assess its acute oral toxicity as per OECD guidelines. A detailed review of relevant Ayurvedic ingredients—such as Loha, Parada, Gandhak, and Kajjali—along with protocols for animal experimentation, provided a strong theoretical base. Standardization of Loha Parpati included organoleptic and physicochemical analyses. The study involved administration of 2000 mg/kg body weight to Wistar rats, with no clinical signs of toxicity observed over a 14-day period. The findings support the safety of Loha Parpati at the tested dose, affirming the role of traditional preparation methods in ensuring its non-toxic nature.*

Keywords: Loha Parpati, Acute oral toxicity, Rasaushadhi, OECD guidelines, Wistar rats

I. INTRODUCTION

Ayurveda has gained renewed interest both in India and around the world, as people turn toward holistic and traditional systems of healing. With this global revival, however, comes the responsibility to validate Ayurvedic formulations through modern scientific standards, particularly when they include potent ingredients like metals.

One of the eight core branches of Ayurveda is *Agadtantra*, which deals with toxins and their management. It describes the effects and treatment of poisons derived from animals, plants, and minerals—many of which are also used therapeutically in processed forms.[1] *Loha Parpati* is one such classical preparation that combines purified forms of iron (*Loha*), mercury (*Parad*), and sulfur (*Gandhak*) to treat a range of disorders, especially digestive conditions like *Grahani* and systemic illnesses such as anemia and jaundice.[2]

Despite its use and therapeutic reputation, there remains a critical knowledge gap regarding the safety of Loha Parpati. Ayurvedic medicines are often assumed to be inherently safe due to centuries of usage. However, concerns raised in international literature about the presence of heavy metals in some Ayurvedic products have highlighted the need for formal toxicological evaluation.[3] These concerns underscore the importance of distinguishing between contaminated products and those where metals are deliberately included—albeit in detoxified, purified forms according to Ayurvedic processing methods.

Given this context, it is now considered essential that multi-ingredient Ayurvedic formulations undergo structured toxicity testing before being clinically evaluated. Loha Parpati, which contains Shuddha Parada (mercury) and Shuddha Loha (iron) as key ingredients, falls into this category. To date, no published research has been found on the acute oral toxicity of Loha Parpati.

This study therefore aims to evaluate the acute oral toxicity of Loha Parpati in Wistar rats using the OECD 423 Acute Toxic Class Method. The findings will help establish its safety profile, determine its therapeutic window, and support responsible integration of traditional formulations into modern healthcare practices.

OBJECTIVE:

Primary Objective- To study the acute oral toxicity class of Loha Parpati in Wistar Rats.

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Secondary Objective-

1) To evaluate the LD50 cut off value of Loha Parpati.
2) To study the Loha Parpati from different text books.

II. REVIEW OF LITERATURE

Loha Parpati is a traditional Ayurvedic medicine known for treating digestive and systemic disorders like *Grahani*, *Pandu*, and *Kamala*. It's made using a unique method called *Parpati Kalpana*, where purified mercury, sulfur, and iron ash are combined, heated, and cooled into thin flakes.[4][5]Ayurvedic texts praise its effectiveness, but modern science calls for careful evaluation—especially because it contains heavy metals.

Even though Ayurvedic processes are designed to detoxify these ingredients, concerns remain about their safety, particularly with mercury and iron, which can cause serious health issues if not properly processed or dosed. That's why it's important to test such formulations through controlled scientific methods.

To ensure safety, animal studies are commonly used. In this study, acute oral toxicity is tested in rats following international OECD Guideline 423. The process helps identify how much of the drug is safe to use and whether any harmful effects occur.[6][7] While these tests provide early safety data, they are just one step in ensuring that traditional remedies like *Loha Parpati* can be used safely and effectively in modern healthcare.

III. MATERIALS AND METHODS

Ethical Approval: The animal study was thoroughly reviewed and approved by both the Institutional Ethical Committee (IEC) and the Institutional Animal Ethical Committee (IAEC) at M.U.H.S. Nashik. This process ensured all ethical guidelines were met before the study began.

Drug Collection: The necessary raw materials for preparing Loha Parpati, including Shuddha Parad, Shuddha Gandhaka, and Loha Bhasma, were sourced from GMP-certified pharmacies to ensure quality and safety.

Preparation and Standardization: The preparation of Loha Parpati followed the traditional methods outlined in *Rasaratnakar*, with all steps verified by a renowned laboratory. To ensure the quality and consistency of the drug, it underwent rigorous standardization, including organoleptic tests (such as appearance, color, and odor) and physicochemical analysis to check pH, ash content, and extractable substances (both alcohol and water-soluble).

Toxicity Study Materials: Wistar rats were used for the acute toxicity study. Before dosing, these rats were acclimatized in the lab for seven days to ensure they met health and weight criteria.

Animal Welfare: The study adhered strictly to the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), ensuring proper animal care throughout. The rats were housed in clean cages with ample space and were provided with commercial food and fresh water.

Study Design:

Step 1: The first group of three female rats was fasted overnight and then given 300 mg/kg of Loha Parpati (diluted in carboxymethyl cellulose). After dosing, they were observed closely for any signs of toxicity for 14 days.

Step 2: Following successful results from Step 1, the same dose was administered to another set of three rats, and they were observed for the same 14-day period.

Step 3: Once the 300 mg/kg dose was confirmed to be safe, a higher dose of 2000 mg/kg was given to three new rats, again following overnight fasting. They were observed for 14 days as well.

Step 4: The safety of the 2000 mg/kg dose was confirmed by repeating the procedure with another set of rats.

Instruments and Materials: A variety of laboratory equipment was used throughout the study, including syringes, needles, weighing scales, and centrifuge tubes, ensuring accurate preparation and dosing for the toxicity assessment.

IV. OBSERVATION AND RESULT

General Monitoring :

The animals were carefully monitored at specific intervals after dosing—30, 60, 120, 180, and 240 minutes. After that, daily observations were continued for 14 days. The duration of monitoring was flexible, depending on the occurrence of

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any toxic reactions, their onset, and recovery times. Any signs of toxicity were recorded systematically for each animal using a daily observation format.

Clinical Signs and Symptoms:

We observed the animals for any changes in the following areas:

Skin, fur, eyes, and mucous membranes.

Respiratory and circulatory systems, as well as autonomic and central nervous functions.

Somatomotor activity and behavior.

Special attention was paid to signs like tremors, convulsions, salivation, diarrhea, lethargy, excessive sleepiness, or even coma.

Body Weight Monitoring:

Body weights were recorded at three points:

Day 0 (before administration, after fasting).

Day 7 and Day 14 after treatment, or at the time of death, if applicable.

Necropsy and Pathology:

At the end of the study, all animals underwent a thorough necropsy. The focus was on detecting any abnormalities in body openings and major organs, including the liver, lungs, kidneys, ovaries, adrenal glands, spleen, pancreas, heart, and brain.

Clinical Observations:

Group I - 300 mg/kg:

No mortality was observed.

The animals showed no signs of toxicity immediately after dosing and appeared normal during the first four hours.

Daily observations over 14 days revealed no signs of intoxication.

Group II - 300 mg/kg:

No mortality was observed.

No immediate clinical signs of toxicity appeared, and animals seemed perfectly normal within the first few hours after dosing.

They showed no signs of intoxication during daily checks up to 14 days.

Group III - 2000 mg/kg:

No mortality occurred.

Immediately after dosing, the animals did not show any signs of toxicity, and they appeared normal for the first four hours.

No signs of intoxication were recorded in the daily observations through Day 14.

Group IV - 2000 mg/kg:

There were no fatalities.

The animals appeared normal up to four hours after dosing, with no signs of toxicity observed.

No symptoms of intoxication were noted during daily checks throughout the 14-day period.

Mortality Results :

A total of twelve female rats were tested at two different dose levels, and Loha Parpati did not cause any fatalities at either 300 mg/kg or 2000 mg/kg.

Step	Dose (mg/kg)	No. of Treated Rats	Terminally Sacrificed	Found Dead (X)
1	300	3	3	0
2	300	3	3	0
3	2000	3	3	0
4	2000	3	3	0
Total	-	12	12	0

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Interpretation of Results:

GHS Classification Based on LD50:

According to the Globally Harmonized System (GHS), chemicals that cause acute toxicity are classified into categories based on their LD50 values. Here's how it breaks down:

LD50	GHS Category
>0-5 mg/kg	Category 1
>5-50 mg/kg	Category 2
>50-300 mg/kg	Category 3
>300-2000 mg/kg	Category 4
>2000-5000 mg/kg	Category 5

Based on the OECD Guidelines, 423, and the conditions of the study, the LD50 of Loha Parpati was found to be in GHS Category 5, with a cut-off value at 5000 mg/kg body weight.

V. DISCUSSION

This study aimed to evaluate the acute toxicity of Loha Parpati, an Ayurvedic rasaushadhi preparation consisting of purified mercury (Parad), sulfur (Gandhak), and iron (Loha), which has been used in Ayurveda for a variety of health conditions, including respiratory and digestive disorders. Despite its historical use, concerns over the presence of heavy metals in these formulations have led to restrictions in certain regions, primarily due to safety concerns. Therefore, it is essential to establish the safety profile of such preparations, particularly regarding their lethal dose.

The study followed OECD 423 guidelines for acute oral toxicity in Wistar rats. The rats were administered Loha Parpati in doses up to 2000 mg/kg body weight. No mortality or clinical signs of toxicity, such as behavioral changes, salivation, diarrhea, convulsions, or death, were observed throughout the study. The rats showed consistent weight gain during the study period, indicating the absence of acute toxic effects. Furthermore, necropsy results revealed no significant pathological changes in the organs, further confirming the safety of the preparation.

Loha Parpati, being an Ayurvedic formulation, undergoes a purification process known as Shodhana, followed by incineration (Maran).[8][9] These methods are intended to reduce the toxicity of the metals and enhance their absorption and therapeutic effects at the cellular level.[10][11] The fine particle size achieved through this purification process aids in the easy absorption of the metals and ensures their safe excretion from the body.[12]

The results of this study indicate that Loha Parpati is safe at therapeutic doses, supporting its continued use in Ayurvedic medicine. By demonstrating the safety of this preparation, the study helps build confidence in Ayurvedic rasaushadhi formulations, particularly in the context of their heavy metal content. This is crucial for their global acceptance and integration into modern healthcare practices.

However, while the acute toxicity study suggests safety at the administered doses, further investigations into long-term toxicity and potential drug interactions are recommended. These studies will provide additional insights into the comprehensive safety profile of Loha Parpati and similar Ayurvedic rasaushadhi formulations.

VI. CONCLUSION

Loha Parpati is safe at doses below 2000 mg/kg, with no toxicity observed in animal studies. Its Ayurvedic preparation methods, like Shodhana and Marana, ensure safety despite its metallic content. Proper dosage guidelines are established, but further research on toxicity and comparisons between self-prepared and market samples is needed to refine its safety profile.

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