

RESEARCH ARTICLE

Acute Oral Toxicity Study of “*Tamra Parpati*” in Wistar Rats by Acute Toxic Class Method

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ABSTRACT

Introduction- *Agad Tantra* is a branch of *Ayurveda* which deals with poison identification and treatment, including natural and artificial toxins. *Tamra Parpati*, a *Rasakalpa* formulation containing metals and toxic substances, requires toxicity assessment for safe use. The research aims to determine the toxicity class of substance and its safe dosage. Acute oral toxicity study of *Tamra Parpati* has not been done before in *Agad Tantra*. **Review of Literature-** *Tamra Parpati* contains two metal contents as *Parad* and *Tamra Bhasma*, one chemical compound *Gandhak* and one poisonous drug *Vatsanabh*. For this study acute toxic class method given in OECD guideline 423 is used. **Materials and Methods-** *Tamra Parpati* is prepared by procedure mentioned in *Yogaratanakar Kasachikitsa Adhyaya*. *Tamra Parpati* has potentially toxic ingredients, undergoes acute oral toxicity testing in Wistar rats using the Acute Toxic Class Method. The study follows a stepwise procedure to assess the safety profile of the formulation. **Result & Discussion-** The results of the study showed no toxicological signs, symptoms, or mortality at doses of 300 mg/kg and 2000 mg/kg. *Tamra Parpati* was classified as non-toxic (GHS category 5) with oral LD₅₀ cut off value at 5000 mg/kg body weight, indicating its potential safety for use.

Key Words *Tamra Parpati, Toxicity, Acute Oral Toxicity, Acute Toxic Class, OECD Guidelines*

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INTRODUCTION

One of the oldest and most popular traditional Indian medical systems is *Ayurveda*. Unexpectedly many subjects are covered by the timeless knowledge of *Ayurveda*. There are eight branches of *Ayurveda*. Toxicology or *Agad Tantra* is one of the eight clinical specializations of *Ayurveda* since thousands of years. *Agad Tantra* deals with poison identification, poison types

from the plant, animal and mineral kingdoms, as well as synthetic poisons and how to treat them. Snake bites, agricultural toxins, toxic metals and minerals, and other plant or animal bites can all result in poisoning. The *Agad Kalpas*, which are used to treat a variety of ailments, including poisoning instances, are also explained by *Agad Tantra*. Natural sources including plants, animals, and minerals provide the drugs utilized in *Ayurvedic* medicine. Because of their

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exceptional properties, the metallic and mercurial compounds known as *Rasaushadhis* are at the top of the list of *Ayurvedic* compositions.

Even if *Rasaushadhis* are being utilized after *Shodhan*, we should be aware of their hazardous dosages for patient safety, as well as dosage and frequency restrictions. Comprising *Tamra*, *Parad*, *Gandhak* and *Vatsanabh*, *Tamra Parpati* is also a *Rasakalpa*. All contents are used in *Kalpa* after their *Shodhan* procedure. *Tamra Parpati* is used to treat various diseases in *Ayurveda*, but its acute oral toxicity has not been thoroughly studied.

To bridge the knowledge gap, this study will conduct an acute oral toxicity assessment using acute toxic class method, in accordance with OECD guideline 423.

REVIEW OF LITERATURE

The popularity of *Ayurvedic* medicines is rising, leading to greater interest in traditional treatment approaches and *Kalpas*. *Tamra Parpati*, a formulation mentioned in several *Ayurvedic* texts, including *Yogaratanakar*, *Rasaratnakara*, *Rasendra Sara Sangraha*, *Bhruhat Nighantu Ratnakara*, *Siddhayoga Sangraha*, *Bharat Bhaishajya Ratnakar*, etc. *Parpati* contains two metal contents as *Parad* (Mercury) and *Tamra Bhasma* (Copper), one chemical compound *Gandhak* (Sulfur) and one poisonous drug *Vatsanabh* (*Aconitum ferox*).

Parad is *Snigdha*, *Sara*, *Guru Gunatmaka* and *Tridoshaghna*. It acts on *Krumi*, *Kushtha* and

Vataroga and *Vyadhiprabhavakara*¹. *Gandhaka* is *Ushna*, *Sara Gunatmak* functions as *Dipana*, *Pachana*, *Vishahara*, *Rasa Shoshana*, *Bala-Virya Vardhaka* and *Rasayana*². The *Tamra Bhasma* is *Ushna*, *Garahara* and capable in relieving the *Yakruta-Plihodar*, *Krumishool*, *Grahani*, *Arsha*, *Amlapitta*³. *Vatsanabha* is a *Rukshna*, *Tikshna*, *Yogavahi*, *Laghu*, *Vyavayi*, *Vikasi* and performs the *Vedanasthapana*, *Shothahar* and *Dipana Karya* as well as impacting on the *Tridosha*⁴.

Acharya Charak stated that “*Visha* is of *Tikshna Guna*, if used properly, it becomes a good medicine and medicine acts like poison if used wrongly”⁵. This underscores the importance of toxicity evaluation. Studies have shown that toxicity testing is crucial for evaluating novel medications, including *Ayurvedic* formulations.

The acute toxic class method, recommended by OECD guideline 423, provides a stepwise approach to assessing acute oral toxicity. This method aims to classify substances based on their toxicity while minimizing animal usage. A review of existing literature reveals that *Tamra Parpati*'s toxicity profile has not been thoroughly studied. Given its complex composition and potential therapeutic applications, further research is necessary to evaluate its safety and efficacy. This review highlights the need for standardized toxicity testing methods to ensure the safe use of *Tamra Parpati* and other *Ayurvedic* medicines in clinical practice.

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AIM

To study the acute oral toxicity of *Tamra Parpati* in Wistar rats by acute toxic class method.

OBJECTIVES

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

RESEARCH QUESTION- Whether *Tamra Parpati* shows acute oral toxicity in Wistar Rats?

HYPOTHESIS

- Null Hypothesis (H₀) - *Tamra Parpati* does not show acute oral toxicity in Wistar Rats.
- Alternate Hypothesis (H₁) - *Tamra Parpati* shows acute oral toxicity in Wistar Rats.

ETHICAL CLEARANCE

The proposed animal study was reviewed and approved by Institutional Ethical committee (IEC) and Institutional Animal Ethical Committee (IAEC) and Maharashtra University of Health Sciences (M. U. H. S.), Nashik. The animal study was started after the approval from chairman of ethical committee of M.U.H.S. Nashik.

MATERIALS & METHODS

A] Drug Preparation- *Tamra Parpati* is prepared by the classical method as explained in *Yogaratanakar Kasachikitsa Adhyaya*⁶.

Material- The raw material was sourced from a GMP-certified pharmacy.

Table 1 List of raw material for preparation of *Tamra Parpati*

Ingredients	Quantity
<i>Shuddha Parad</i>	3 Parts
<i>Shuddha Gandhak</i>	3 Parts
<i>Shuddha Tamra Bhasma</i>	3 Parts
<i>Shuddha Vatsanabh Churna</i>	1 Part
<i>Goghruta</i>	As per required
<i>Arka leaf</i>	As per required

Method-

- 1) *Shuddha Tamra Bhasma*, *Shodhit Parad*, *Shodhit Gandhak* and *Shodhit Vatsanabh Churna* were taken in *Khalvayantra* and *Kajjali* was prepared by trituration.
- 2) *Kajjali* was molten in *Grutalipta Palikayantra*. The molten *Kajjali* was poured on *Arka* leaf smeared with *Goghruta*, placed over cow dung.
- 3) It was covered then with another *Arka* leaf and pressed gently.
- 4) Allowed it to cool, leaves were taken off and flakes of *Tamra Parpati* were collected and preserved.
- 5) Then obtained *Tamra Parpati* was grinded to obtain its powder form.



Figure 1: Preparation of *Tamra Parpati*

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Standardization of drug-

Tamra Parpati prepared was standardized from renowned laboratory. It includes analytical study report of *Tamra Parpati*.

Table 2 Parameters of standardization

Examinations	Parameters Assessed
Organoleptic	Appearance, Colour, Odour, Taste
Physicochemical	Loss on Drying, pH, Ash value, Water-Soluble Extractives, Alcohol-Soluble Extractives, Density

B] Acute oral toxicity study⁷ -

Material-

Table 3 List of study instruments

Test Components	<i>Tamra Parpati</i>
Vehicle	Emulsion / Suspension
Syringes	1 ml. insulin syringe
Needles	Aspirator needle
Centrifuse Tubes	Plastic graduated 10 ml. tubes
Weighing balance	SKNOL make
Mixer	Vorter Mixer
Butter Paper	Small pieces

Method-

1. Preparation of animals-

Table 4 Preparation of animals

Species	Rats
Strain	Wistar rats
Source	Crystal Biological Solutions
Sex	Female
Body weight range	80-120 gm.
Identification	Identification mark to animals and cages
No. of animals	12
Acclimatization	The rats were Acclimatized at test environment for 7 days.
Environmental conditions	Testing room temperature maintained between 22 ± 30C, relative humidity 55 ± 5 % and illumination cycle set to 12 hours light and 12 hours dark
Accommodation	Three animals per cage housed in polypropylene cage and SS cage top cages, with facilities for food and water bottle, and bedding of clean paddy husk.
Diet	Commercial Pelleted food supplied by Nutrivet Pvt. Ltd., Pune, was provided ad libitum during acclimatization and during

the study.

Water

Potable water passed through 'RO Unit' provided ad libitum in Polypropylene bottles with stainless steel sipper tubes.

Dosing

Oral

1. Study Design-

Twelve female Wistar rats were selected for the study and allowed to acclimatize for period of 7 days prior to dosing. During this period animals were observed daily for clinical signs. The health and weight criteria were successfully met by all animals.

Step 1: A dose of 300mg/kg body weight of *Tamra Parpati*, diluted in carboxy methyl cellulose, was administered orally to three overnight fasted female rats (Group 1). The rats were deprived of food overnight before dosing and 2 hours after the dosing. Water was allowed ad libitum throughout the study period. All the animals were observed for 14 days after dosing.

Step 2: Three overnight fasting female rats (Group 2) were administered the *Tamra Parpati* diluted in carboxy methyl cellulose at the dose of 300 mg/kg body weight, after animals were found to be safe in step 1. The rats were deprived of food overnight before dosing and 2 hours after the dosing. Water was allowed ad libitum throughout the study period. All the animals were observed for 14 days after dosing.

Step 3: After confirmation of safety at 300 mg/kg at previous step, 3 overnight fasting female rats (Group 3) were again administered with the *Tamra Parpati* diluted in carboxy methyl cellulose at the dose of 2000mg/kg body weight.

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The rats were deprived of food overnight before and 2 hours after the dosing. Water was allowed ad libitum. All animals were observed for 14 days after dosing.

Step 4: Step 3 was again repeated in 3 overnight fasting female rats (Group 4) after confirmation of the safety at 2000 mg/kg of dose.



Figure 2 Animal Experiment

OBSERVATION AND RESULT

Standardization of *Tamra Parpati*-

Table 5 Standardization study report

Test	Result
Organoleptic examination	
Appearance	Fine Powder
Colour	Black
Odour	Unpleasant
Taste	Bitter
Physicochemical analysis	
Loss on Drying	0.64
pH	7.8
Ash	41.02 %
Alcohol Soluble Extractive	2.15 %
Water Soluble Extractive	2.4 %
Density	1.69

Acute oral toxicity test-

- Animals were observed individually for first 30, 60, 120, 180 and 240 minutes after dosing, with special attention and once daily thereafter, for a total of 14 days.
- Clinical signs and symptoms-

- All animals were observed for the following signs- Changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, behaviour pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

- The *Tamra Parpati* caused no mortality. All the animals did not show any clinical signs of toxicity immediately after dosing and appeared normal up to four hours and showed no clinical signs of intoxication at daily observations up to 14 days in all groups of 300 mg/kg and 2000 mg/kg body weight.

- **Body Weights-**

- The body weights were recorded on test day 0 (pre-administration - fasting weight) and on days 7 and 14 post dosing.

- Normal Body weight gain was observed during 14 days observation period and there were no any signs of toxicity considering weight gain.

- **Gross Necropsy-**

- All animals were subjected to gross necropsy. In gross necropsy the animals were observed at all the body openings, opened up and observed it with naked eye for any alterations in normal body organs. At this point major organs like liver, lungs, ovaries, kidneys, adrenal gland, spleen, pancreas, heart, brain etc. were observed.

- *Tamra Parpati* did not cause morality in the female rats treated at the dose of 300mg/kg and 2000 mg/kg body weight.

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- The LD₅₀ value of '*Tamra Parpati*', according to the OECD Guideline 423 and under provided laboratory conditions to Wistar rats, was found to be in Globally Harmonised System (GHS) Category 5, > 2000 - 5000 mg/kg body weight, with a LD₅₀ cut off at 5000 mg/kg body weight.

DISCUSSION

Ayurvedic herbal and herbo-mineral preparations are utilized for disease treatment due to their perceived lack of side effects. In this study, *Tamra Parpati* was prepared according to the procedure outlined in *Yogaratanakara*, using *Shuddha Parad*, *Shuddha Gandhaka*, *Tamra Bhasma* and *Shuddha Vatsanabh Churna* in a 3:3:3:1 ratio. Each component of *Tamra Parpati* possess distinct characteristics.

Tamra Parpati is traditionally employed to treat various diseases including *Yakshma*, *Sannipataja Vikaras*, *Pandu*, *Shool*, *Dadru*, *Prameha*, *Kushtha* and *Vata-Pitta* diseases. To establish the safety and global acceptability of *Rasakalpas* like *Tamra Parpati*, it is essential to determine their safe dosage.

The study investigated the acute oral toxicity of *Tamra Parpati* in Wistar rats using the Acute Toxic Class Method, as given in OECD guideline 423. Twelve female rats were tested at two dose levels: 300 mg/kg and 2000 mg/kg body weight. During the 14-day observation period, the rats exhibited normal body weight gain, and no toxicological signs or symptoms were observed.

Tamra Parpati did not cause mortality in the female rats treated at either dose level. At the end of the test, all surviving animals were humanely killed and subjected to gross necropsy, which revealed no abnormalities or pathological changes.

Based on the results of the study and the Globally Harmonized System (GHS) classification of chemicals causing acute toxicity, the LD₅₀ value of *Tamra Parpati* was determined to be in GHS category 5 (>2000-5000 mg/kg body weight), with an LD₅₀ cut-off value at 5000 mg/kg body weight, indicating that *Tamra Parpati* is non-toxic for use.

CONCLUSION-

- *Tamra Parpati* has been found to be safe in Wistar rats at a dose of 2000 mg/kg body weight, as per OECD guideline 423, under controlled laboratory conditions. This indicates that oral administration of *Tamra Parpati* at doses below 2000 mg/kg body weight, whether as a single dose or multiple doses within a 24-hour period, is unlikely to result in adverse effects.
- The safety of *Tamra Parpati*, despite its metallic and toxic constituents, is relies on precise preparation, including proper *Shodhan* and *Maran* procedures. The findings of this study can provide valuable insights for determining dosage and regimen.
- Standardization and scientific validation are essential for supporting the controlled therapeutic use of *Tamra Parpati*. Nevertheless, additional

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research is required to assess sub-acute and chronic toxicity, conduct clinical trials, and establish the long-term safety and fatal dose of *Tamra Parpati*.

- This further research will yield a more comprehensive understanding of safety profile of *Tamra Parpati*, thereby facilitating its safe and effective use.

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