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Acute Oral Toxicity Study Of Vetal Rasa In Wistar Rats By Acute Toxic Class Method

Dr. Shivani V. Kotmire [1]and Dr. Manohar Khankhane [2]

MD (Scholar) Department of Agadtantra Avum Vidhivaidyaka[1]

Professor and H.O.D. PG Diploma in Agadtantra Avum Vidhivaidyaka[2]

MAM's Sumatibhai Shah Ayurved Mahavidyalaya, Hadapsar, Pune, India

ABSTRACT

In Ayurveda, Rasaushadhis (Herbo-mineral formulations) hold significant therapeutic value, yet their safety requires scientific validation. Vetal Rasa, described in Rasendra Sara Sangraha, is used in Sannipataja Jwara and critical conditions. Comprising Shuddha Parada (purified mercury), Shuddha Gandhaka (sulphur), Shuddha Haratala (orpiment), Shuddha Vatsanabha (aconite), and Maricha (black pepper), this formulation necessitates toxicity assessment. This study evaluated its acute oral toxicity in Wistar rats following OECD 423 guidelines. Vetal Rasa was administered at 300 mg/kg and 2000 mg/kg, with a 14-day observation period. No Upadrava Lakshanas (adverse effects), mortality, or pathological changes were noted. As per GHS classification, it falls under Category 5 (LD50 > 2000 mg/kg), indicating low toxicity. These findings support its safety within Yogya Matra (appropriate dosage), though further studies on chronic toxicity are needed to establish its long-term effects and regulated clinical application.

Keywords: Toxicity Assessment, Vetal Rasa, Rasaushadhi, Acute toxicity, Yogya Matra, OECD Guidelines

INTRODUCTION

Ayurveda, the eternal science of life, is well-established in the realm of holistic healing, with its divine purpose being the protection of health in the healthy and the alleviation of disorders in the diseased. Among the eight sacred branches of Ayurveda, Agadatantra, the science of toxicology, deals with the knowledge of various poisons, their signs and symptoms, and their effective elimination through divine and herbal formulations.[1] The term Agada signifies that which annihilates the harmful effects of toxins, restoring equilibrium within the body.[2]

In the vast ocean of Rasa Shastra, herbo-mineral formulations hold a significant place, revered for their swift action, potency in minute doses, and the ability to cure even the most grievous ailments. Among them, Vetal Rasa, a sacred Kalpa enshrined in *Rasendra Sara Sangraha*[3], is prescribed for the management of Sannipataja Jwara, Moha, and other Atiyayik Avasthas. Composed of Parada (mercury), Gandhaka (sulphur), Haratala (arsenic), Vatsanabha (aconite), and Maricha (black pepper), this formulation is a potent Rasayana; yet, due to the presence of toxic Dravyas, a thorough Vishamapaka and toxicity assessment is paramount to ascertain its safety for therapeutic use.

Thus, to establish the Yogya Matra (safe dosage) and avoid Upadrava (adverse effects), a scientific exploration of its acute oral toxicity is essential. Employing the Acute Toxic Class Method, a standardized protocol for toxicity evaluation, this study endeavors to assess the safety profile of Vetal Rasa in Wistar rats.

Vd. Satyarth Prakash, in his translation of Rasendra Sara Sangraha by Sri Gopal Krishna, has described Vetal Rasa in the Jwarachikitsa Adhyaya, detailing its composition, preparation, and therapeutic applications. While the individual toxic effects of Parada, Gandhaka, Haratala, and Vatsanabha—the core ingredients of this Kalpa—have been studied previously, no comprehensive scientific research on the acute oral toxicity of Vetal Rasa has been documented. To the best of my knowledge, no prior study has explored Vetal Rasa from the perspective of Agadatantra.

Thus, to establish the Yogya Matra (safe dosage) and avoid Upadrava (adverse effects), a scientific exploration of its acute oral toxicity is essential. Employing the Acute Toxic Class Method, a standardized protocol for toxicity evaluation, this study endeavors to assess the safety profile of Vetal Rasa in Wistar rats. The outcome shall illuminate its Aushadhiyata (therapeutic utility) and safety, bridging the sacred wisdom of Ayurveda with the rigor of modern scientific validation.

AIMS AND OBJECTIVES

Aim: To Study Acute Oral Toxicity of "Vetal Rasa" In Wistar Rats by Acute Toxic Class Method.

Primary Objective: To study the acute oral toxicity class of Vetal Rasa in wistar rats.

RESEARCH QUESTION: Whether Vetal Rasa shows acute oral toxicity In Wistar Rats?

HYPOTHESIS

- Null Hypothesis (H0) -Vetal Rasa does not shows acute oral toxicity in Wistar Rats.
- Alternative Hypothesis (H1)- Vetal Rasa shows acute oral toxicity in Wistar Rats.

REVIEW OF LITERATURE

Ayurvedic Perspective on Visha (Poison)

The Ayurvedic perspective on Visha (poison) describes it as a substance that spreads rapidly within the body, disturbing the equilibrium of Doshas (Vata, Pitta, Kapha) and affecting the Dhatus (tissues) and Malas (excretory products).[4] This results in severe physiological disruptions and can lead to Pranahani (loss of life). The term Visha is defined as a substance that causes distress and systemic imbalance. Ayurveda emphasizes that while poisons have the potential to cause harm, they can also be used therapeutically when purified (Shodhana) and administered in controlled doses (Matra).

The classical texts describe the unique properties of Visha, including its quick action, deep penetration, rapid spread, and inability to undergo normal digestion.[5] Due to these properties, Visha has both therapeutic and destructive potential, necessitating its careful processing before medicinal use.

Vetal Rasa and Toxicity Considerations

It is composed of Shuddha Parada (Mercury), Shuddha Gandhaka (Sulfur), Shuddha Haratala (Orpiment - Arsenic Trisulfide), Shuddha Vatsanabha (Aconite), and Maricha (Black Pepper). This formulation is traditionally used in conditions such as Sannipataja Jwara (severe fevers), Moha (delirium, unconsciousness), and Atyayik Avastha (critical conditions).[3] However, because it contains highly toxic ingredients, its safety profile must be assessed through scientific validation and toxicity studies before clinical application.

Modern Review of Poison

From a modern perspective, poisons are broadly defined as substances that, when introduced to the body, cause chemical and physiological disturbances leading to disease or death.[6] They are categorized based on their effects, including corrosive poisons (strong acids and alkalis), irritant poisons (arsenic, phosphorus, lead, mercury), and systemic poisons, which affect specific organ systems such as the nervous, cardiovascular, and respiratory systems.[7] Medicolegal classification includes suicidal, homicidal, accidental, stupefying, abortifacient, cattle poisons, and food poisoning agents.[8]

Toxicity Study

Vetal Rasa contains potent toxic ingredients that require extensive purification and controlled administration to minimize harmful effects. Conducting scientifically validated toxicity studies following OECD guidelines is essential to establish its safety profile and determine its therapeutic applicability. This research will provide a scientific basis for the controlled use of Vetal Rasa in Ayurvedic treatment protocols, ensuring both efficacy and safety.

MATERIALS AND METHODS

1. Ethical Clearance

The study was reviewed and approved by the Institutional Ethical Committee (IEC) and Institutional Animal Ethical Committee (IAEC) before submission to M.U.H.S. Nashik. Approval was obtained from the chairman of the ethical committee before commencing the study.

2. Collection of Raw Drugs

The raw materials were sourced from a GMP-certified pharmacy.

Ingredients	Quantity
Shuddha Parada	1 Part
Shuddha Gandhaka	1 Part
Shuddha Haratala	1 Part
Shuddha Vatsanabha	1 Part
Marich	1 Part

Pharmaceutical Preparations

Drug Preparation:

Equipment Used

- Khalvayantra (Grinding Apparatus)
- Weighing Machine

Preparation Method

- Kajjali preparation – Triturated Parada and Gandhaka in Khalvayantra until it passed standard tests (Nishchandratva).
- Added Shodhit Haratala, Shodhit Vatsanabha Churna, and Marich Churna to Kajjali.
- Further trituration until a homogeneous mixture was obtained, forming Vetal Rasa.

Standardization of Drug

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

Loss on Drying

- Measures moisture and volatile components.
- Sample was dried at 105°C for 2 hours until a constant weight was obtained.

pH Measurement

- A 10% solution of the sample was prepared in distilled water.
- Measured using a pH meter calibrated at 4.2 and 9.0.

Total Ash Content

- Determines inorganic matter in the drug.
- Sample was incinerated until complete removal of carbon.

Water-Soluble & Alcohol-Soluble Extractives

Parameter	Method
Water-Soluble	Soaked in chloroform-saturated water, stirred, filtered, evaporated, and weighed.
Alcohol-Soluble	Macerated in 95% methanol, filtered, evaporated, and weighed.

Authentication and Standardization

Authentication: Verified by a renowned laboratory before use.

Preparation Method: Followed Rasendra Sara Sangraha.[3]

In-Vivo Toxicity Study

Ethical Approvals & Guidelines:

- Conducted under OECD 423 guidelines.
- Approved by CPCSEA & Institutional Animal Ethics Committee (IAEC) of Crystal Biological Solutions.
- Ensured animal welfare as per CCSEA and The Gazette of India (1998).

Materials & Equipment

Materials	Equipment
Vetal Rasa (Test Compound)	1 mL Insulin Syringe
Vehicle (Emulsion/Suspension)	Aspirator Needle
Butter Paper	Plastic Graduated Centrifuge Tubes (10 mL)
-	Weighing Balance (SKNOL)
-	Vortex Mixer

Preparation of Animals

Parameter	Details
Species & Strain	Wistar Rats
Sex	Female
Body Weight Range	80–120 g
Source	Crystal Biological Solutions
Acclimatization	7 days under controlled conditions
Environmental Conditions	Temperature: 22 ± 3°C
	Humidity: 55 ± 5% Light/Dark Cycle: 12-hour cycle
Housing	3 rats per polypropylene cage with stainless steel cage tops
Diet	Commercial pelleted food (Nutrivet Pvt. Ltd., Pune)
Water	RO-filtered potable water (ad libitum)

Study Design

The toxicity study was conducted in 4 steps using a stepwise approach.

Step	Group	Dose (mg/kg)	Observations
Step 1	Group I	300 mg/kg	Observed for 14 days
Step 2	Group II	300 mg/kg	Reconfirmed safety, observed for 14 days
Step 3	Group III	2000 mg/kg	Observed for 14 days
Step 4	Group IV	2000 mg/kg	Reconfirmed safety, observed for 14 days

- Fasting: All rats were fasted overnight before dosing and for 2 hours post-dosing.
- Water: Provided ad libitum throughout the study period.

OBSERVATION AND RESULTS

Standardization of Vetal Rasa

Organoleptic Properties	
Appearance	Fine Powder
Colour	Greyish Black
Odor	Faint
Taste	Slightly Pungent
Physicochemical Analysis	
Loss on Drying	0.79%
pH	7.8
Ash Content	36.04%
Alcohol-Soluble Extractives	3.33%
Water-Soluble Extractives	3.76%
Density	1.34

2. Acute Oral Toxicity Study in Wistar Rats

Observation Period:

- Animals were monitored at 30, 60,120,180and 240 minutes post-dosing and once daily for 14 days.
- No toxic symptoms were observed in any group.

Clinical Signs:

- No abnormal changes in skin, fur, eyes, mucous membranes, respiration, nervous system, or behaviour.
- No signs of tremors, convulsions, diarrhea, lethargy, coma, or toxicity.

Body Weight Monitoring: Normal weight gain was observed in all groups, with no toxicity-related changes.

Mortality: No deaths recorded in any dose group (300 mg/kg and 2000 mg/kg).

3. Gross Necropsy and Pathology Findings

- No pathological alterations were found in major organs (brain, liver, lungs, heart, kidneys, intestines, spleen, etc.).
- All animals appeared healthy with no internal abnormalities.

4. GHS Classification and LD50 Determination

- According to GHS (Globally Harmonized System) acute toxicity classification, Vetal Rasa falls under Category 5 (LD50 > 2000 - 5000 mg/kg), indicating low toxicity.
- LD50 Cutoff: 5000 mg/kg
- Conclusion: Vetal Rasa is non-toxic at tested doses and safe for therapeutic use.

DISCUSSION

Herbo-mineral formulations play a significant role in Ayurveda, but concerns regarding their toxicity persist, especially for medicines containing heavy metals. Vetal Rasa includes Parada (mercury), Gandhak (sulphur), Haratala (arsenic), and Vatsanabha (aconite), all of which require purification to ensure safety. While traditional methods claim to detoxify these substances, scientific validation is necessary to determine safe dosage limits. This study aimed to assess the acute toxicity of Vetal Rasa to establish its safety profile.

A review of the literature highlighted the pharmacological properties of its ingredients. Mercury is known for its Rasayana (rejuvenating) effects but demands precise purification. Sulfur and arsenic are used in Ayurvedic treatments for skin and metabolic disorders, while aconite, despite its toxicity, is traditionally used in fever and respiratory ailments. The presence of these potent substances necessitates toxicity evaluation, particularly regarding cumulative effects.

The study followed OECD 423 guidelines using the Acute Toxic Class (ATC) method in Wistar rats. Vetal Rasa was prepared as per Rasendrasarasangraha, authenticated, and analysed for its physicochemical properties. Twelve female Wistar rats were tested in four groups with stepwise dosing, starting at 300 mg/kg and escalating to 2000 mg/kg. No adverse effects, mortality, or pathological changes were observed during the 14-day monitoring period.

The results demonstrated that Vetal Rasa did not induce any signs of acute toxicity at both 300 mg/kg and 2000 mg/kg doses. No adverse symptoms such as salivation, diarrhea, convulsions, or coma were noted during the 14-day observation period. Furthermore, there was no evidence of mortality, and body weight progression remained within normal limits. Necropsy findings revealed no gross pathological alterations, indicating an absence of systemic toxicity. The ATC method allowed for classification based on predefined dose levels, aligning with global safety assessment standards.

These findings suggest that Vetal Rasa, when administered in controlled doses, does not exhibit acute toxicity in Wistar rats. While the absence of immediate adverse effects is promising, further chronic toxicity studies are necessary to assess long-term safety and potential cumulative effects. Given the historical concerns surrounding Ayurvedic formulations containing heavy metals, such studies are essential to reinforce their credibility and facilitate wider acceptance in integrative medicine. The results of this study contribute to the scientific validation of Vetal Rasa, supporting its continued therapeutic use within defined safety parameters.

CONCLUSION

The study concludes that Vetal Rasa is safe at doses up to 2000 mg/kg, with no observed toxicity or mortality in treated Wistar rats. Despite its metallic content, purification processes like Shodhana and Marana ensure its safety. Standardization and scientific validation support its controlled therapeutic use, though further studies on sub-acute, chronic toxicity, and clinical trials are needed to establish its long-term safety and fatal dose.

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