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Research Article

# COMPARATIVE ACUTE TOXICITY PROFILE OF *RASAPUSHPA* PREPARED BY CLASSICAL AND CONVENTIONAL METHODS

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**ABSTRACT**

*Rasapushpa*, an Ayurvedic mercurial preparation, is a very potent formulation due to its therapeutic properties but carries potential risks due to the presence of mercury. The acute toxicity of *Rasapushpa*, prepared by two distinct methods- the classical *Valukayantra* method (RPVY) and the conventional Electric Muffle Furnace method (RPEMF)- was assessed in Wistar rats. Using the OECD Test Guideline 423, the study determined the lethal dose ($LD_{50}$) and observed any clinical signs of toxicity following oral administration. **Objectives:** To evaluate the Acute toxicity study of *Rasapushpa* prepared by both methods. **Materials and Methods:** This comparative Acute toxicity study was conducted at the Ribosome Research Centre, Kudsad, Surat, using two samples of *Rasapushpa*: RPVY (classical method) and RPEMF (conventional method). By adopting OECD Test Guideline 423. **Discussion:** *Rasapushpa* prepared by *Valukayantra* (RPVY) exhibited $LD_{50}$ at 300mg/kg body weight, while *Rasapushpa* prepared by Electric Muffle Furnace (RPEMF) showed $LD_{50}$ higher than 2000 mg/kg. **Conclusion:** After human dose conversion, both samples were found safe in the dose range given by *Rasatarangini* for *Rasapushpa* (62.5 to 312.5 mg).

## INTRODUCTION

Pharmacology investigates how drugs interact with biological systems, focusing on their therapeutic effects, side effects, and toxicity. A key aspect of pharmacological safety is the evaluation of acute toxicity, which helps identify harmful reactions following a single exposure to a substance.[1]

*Rasapushpa* is a traditional Ayurvedic formulation that contains mercury, a metal known for its potential toxicity.[2] Due to its hazardous nature, mercury is classified under Schedule E (1) of the Drugs & Cosmetics Act, 1940.[3] *Rasapushpa* is traditionally prepared using *Valukayantra*. It is also prepared by a more modern approach using the Electric Muffle Furnace (RPEMF) nowadays, which has gained popularity due to its ability to provide precise temperature control during preparation.

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There is limited understanding of the relative toxicity of *Rasapushpa*. So, this study aims to assess the acute toxicity of *Rasapushpa* prepared by both the classical *Valukayantra* method and contemporary Electric Muffle Furnace method.

Acute toxicity studies in animals are essential for any pharmaceutical intended for human use. They help determine appropriate dosing for repeat-dose studies and offer initial insights into target organs affected by toxicity. Additionally, these studies can guide the selection of starting doses for phase 1 human trials and provide valuable information related to potential acute overdosing in humans.[4]

Acute toxicity studies are conducted to determine the short-term adverse effects of a drug when administered in a single dose or multiple doses during 24 hours in two mammalian species (one non-rodent).[5] The goal is to determine the safe dosage levels for samples and to compare their toxicological profiles, providing valuable insights into their safety and potential risks for use in clinical settings.

## Preparation of *Rasapushpa*

### I. Procurement of raw materials

*Saindhava* and *Sudha* were procured from Government Ayurvedic Pharmacy, Rajpipla, Gujarat. Fresh *Bhringaraja Panchanga* was procured from local gardens and *Rasona* was procured from the local market of Vadodara, Gujarat. *Ashuddha Kasisa* and *Ashuddha Parada* were procured from local traders of Vadodara and authenticated by comparing them with their classical *Grahya Lakshana*.

### II. Preparation of drug

*Parada Shodhana* was done with *Sudha, Rasona* and *Saindhava* by adopting the reference of *Rasatarangini* (*Taranga* 5/27). *Kasisa Shodhana* was done by *Swedana* in *Bhringaraja Svarasa* by adopting the reference of *Rasatarangini* (*Taranga* 21/230). Three batches of *Rasapushpa* by each method viz., *Rasapushpa* by classical method and *Rasapushpa* by conventional method were prepared for the development of standard manufacturing procedure (SMP) in the pharmaceutical laboratory of Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat. Three batches of *Rasapushpa* were prepared by classical *Valukayantra* method and conventional Electric muffle furnace (EMF) by adopting the reference of Rasatarangini (*Taranga 6*/29-31).

**Figure 1: Preparation method of *Rasapushpa* by *Valukayantra* method**

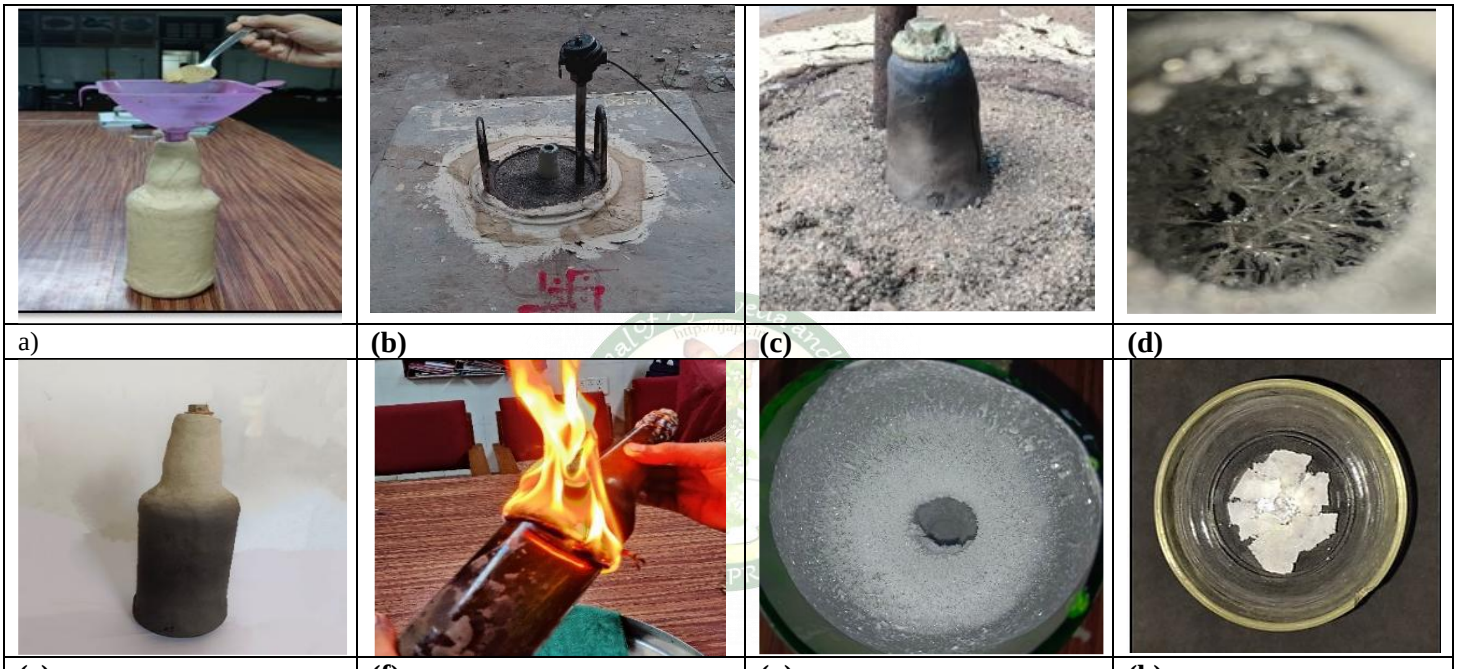
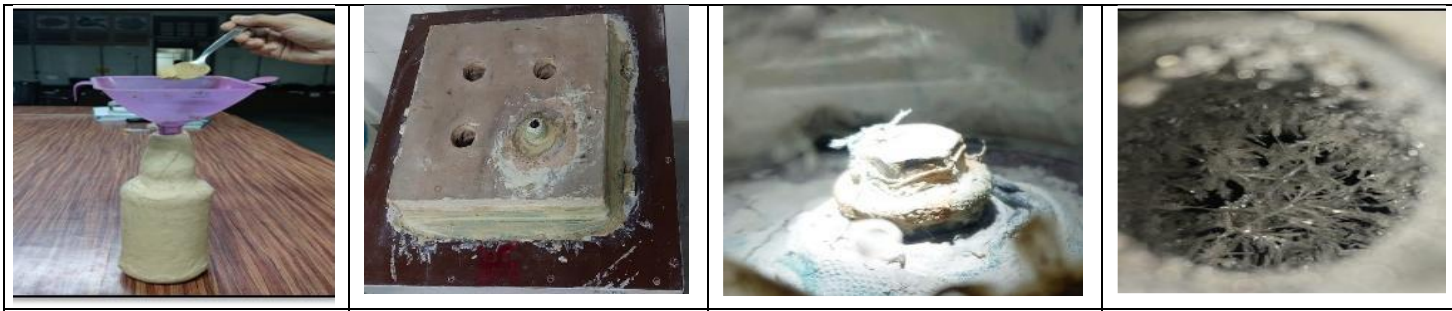
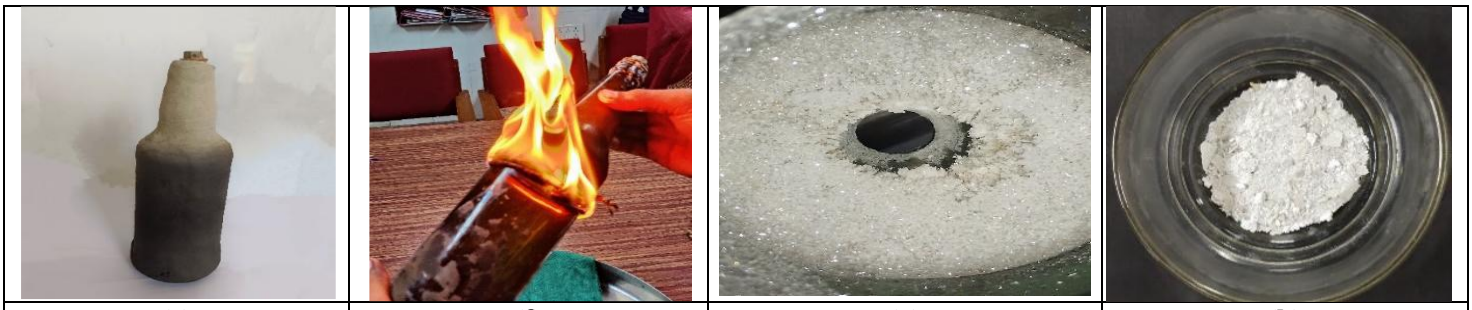



**Figure. 1: (a)** Filling of *Kajjali* into *Kachakupi,* **(b)** Placing of *Kupi* at the centre of *Valukayantra,* **(c)** Loose corking, **(d)** Crystallization of *Rasapushpa* at the neck of *Kupi*, **(e)** *Swangasheeta Kupi* after 6 hours of *Paka,* **(f)** Breaking of *Kupi,* **(g)** Crystallization of *Rasapushpa* inside the *Kupi,* **(h)** Crystalline *Rasapushpa* as a final product

**Figure 2: Preparation method of *Rasapushpa* by EMF method**





| (e) | (f) | (g) | (h) |
|---|---|---|---|

**Figure. 2: (a)** Filling of *Kajjali* into *Kachakupi,* **(b)** Placing of *Kupi* in vertical EMF, **(c)** Loose corking, **(d)** Crystallization of *Rasapushpa* at the neck of *Kupi*, **(e)** *Swangasheeta Kupi* after 6 hours of *Paka,* **(f)** Breaking of *Kupi,* **(g)** Crystallization of *Rasapushpa* inside the *Kupi,* **(h)** Crystalline *Rasapushpa* as a final product

**Toxicity Study Protocol**

**OBJECTIVES**

To evaluate acute toxicity profile of *Rasapushpa* prepared by both methods.

**MATERIALS AND METHODS**

An acute oral toxicity study was conducted at the Ribosome Research Centre located in Kudsad, Surat, Gujarat. The test substances used were two samples of *Rasapushpa*, prepared by different methods.

Sample 1 was RPVY (*Rasapushpa* prepared by classical *Valukayantra* method) and Sample 2 was RPEMF (*Rasapushpa* prepared by the conventional Electric Muffle Furnace method). Both preparations were stored at room temperature in a solid, whitish form prior to testing. The study followed the OECD Test Guideline 423: 2001, which outlines the procedures for acute oral toxicity testing using the Toxic Class Method. Ethical approval was obtained from the Institutional Animal Ethics Committee (CPCSEA Registration No. 2078/PO/RcBi/S/19/CPCSEA), and the study complied with ethical guidelines for animal care. All procedures were approved by relevant regulatory bodies, including the Food and Drugs Control Administration (FDCA), GLP certification, and the Ministry of AYUSH.

**Test System**

**Table 1: Test system details of acute toxicity study of *Rasapushpa***

| | | |
|---|---|---|
| 1 | Species | Rat (*Rattus norvegicus*) |
| 2 | Strain | Wistar |
| 3 | Sex | Female (Females were nulliparous and non-pregnant. |
| 4 | Age of animal | At least 8 Weeks old and 200-300 g at the start of treatment. |
| 5 | Body Weight | ± 20% of the mean weight of any previously dosed animals. |
| 6 | No. of Animals | 03 Animals per set. |
| 7 | Source | Ribosome Research Centre Pvt. Ltd.<br>(CPCSEA Registration No.2078/PO/RcBi/S/19/CPCSEA) |

The rats were acclimatized in standard laboratory conditions for 5 days before dosing. They were housed in polypropylene cages and maintained at a constant room temperature of 21.4–22.9°C, with relative humidity ranging from 47–64% and a 12-hour light/dark cycle. The animals were provided with standard laboratory feed and fresh potable water ad libitum.

**Dosing and Administration**

The test preparations were administered orally via gavage by using 1% CMC (Carboxy-methyl cellulose) as a vehicle. The initial dose for *Rasapushpa* was set at 300mg/kg body weight. Subsequent doses were adjusted to 50mg/kg for RPVY and 2000mg/kg for RPEMF. The dosing volume was 1mL per 100gm of body weight for all animals. As there was no prior public domain information about these formulations, the starting dose was set at 300mg/kg body weight, with a subsequent higher dose of 2000mg/kg body weight for safety.

**Observations and Monitoring**

Animals were monitored twice daily for signs of mortality or abnormal behaviour. Clinical observations were made at intervals of 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours after dosing, and daily thereafter for 14 days to detect any clinical signs of toxicity. The body weight of each animal was recorded on Days 0, 7, and 14 to track any significant changes. The animals were euthanized using $CO_2$ asphyxiation, and post-mortem examinations were performed to observe any gross pathological changes in the internal organs.

## Results

For RPVY, in Set I (300mg/kg), all three animals died within 4 hours to 4 days post-dose. In Set II (50mg/kg), all three animals survived with no clinical signs, suggesting lower toxicity. Set III (50mg/kg) showed similar results, with no mortality or clinical signs. For RPEMF, in Set I (300mg/kg), one animal died within 4 hours, while the other two survived without any signs of toxicity. In Set II (300mg/kg), all three animals survived and showed no signs of morbidity. In Set III (2000mg/kg) and Set IV (2000mg/kg), no mortality or clinical signs were observed at these high doses, with all animals surviving without observable toxicity.

**Table 2: Mortality Rates**

| Sample | Set | Dose (mg/kg) | Mortality |
|---|---|---|---|
| **RPVY** | I | 300 | 3/3 |
| | II | 50 | 0/3 |
| | III | 50 | 0/3 |
| **RPEMF** | I | 300 | 1/3 |
| | II | 300 | 0/3 |
| | III | 2000 | 0/3 |
| | IV | 2000 | 0/3 |

## Clinical Signs

In RPVY, animals in Set I showed signs of morbidity and death, while no clinical signs were observed in Sets II and III, with the animals remaining healthy. In RPEMF, no clinical signs of toxicity, such as abnormal behaviour, tremors, or lethargy, were observed in any animals across all sets, indicating a favourable safety profile. Body weight measurements were recorded at the start and throughout the study, with no significant weight loss or changes observed in the surviving animals. Body weight taken on Days 0, 7, and 14 showed no significant weight loss, suggesting that the preparations did not cause severe physiological disturbances in the animals.

**Table 3: Body Weight Progression**

| Sample | Set | Dose (mg/kg) | Day 0 (g) | Day 7 (g) | Day 14 (g) |
|---|---|---|---|---|---|
| RPVY | II | 50 | 243.0 | 257.3 | 272.3 |
| | | | 248.4 | 263.4 | 278.6 |
| | | | 239.0 | 254.1 | 261.4 |
| RPEMF | III | 2000 | 217.7 | 232.2 | 249.8 |
| | | | 219.2 | 233.9 | 250.6 |
| | | | 220.8 | 236.0 | 253.8 |

**Table 4: Individual Animal Gross Pathology observations in RPVY group**

| Animal No. | Sex | Days | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| | | Within 30 min | 1 h | 2 h | 3 h | 4 h | | | | | | | | | | | | | | |
| | | | ± 10 minutes | | | | | | | | | | | | | | | | | |
| 1 | F | 1 | 1 | 75 | 75 | 75 | Death | | | | | | | | | | | | | |
| 2 | F | 1 | 1 | 1 | 75 | 75 | Death | | | | | | | | | | | | | |
| 3 | F | 1 | 1 | 1 | 1 | 1 | 75 | Death | | | | | | | | | | | | |
| 7 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 8 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 9 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 13 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 14 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 15 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

- 1= Normal
- F = Female
- 75 = Moribund Status (Being in the state of dying. A diagnosis and decision point based on appropriate clinical judgment, taking into account the severity of the condition, prognosis, potential loss of data, pain and distress).

➢ No clinical sign was observed in any of the animals in Sets I, II, III and IV when treated with the test item *Rasapushpa* using the EMF - Electric Muffle Furnace.

**Table 5: Individual Animal Gross Pathology observations in RPEMF group**

| Animal No. | Sex | Days | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| | | Within 30 min | 1 h | 2 h | 3 h | 4 h | | | | | | | | | | | | | | |
| | | | ± 10 minutes | | | | | | | | | | | | | | | | | |
| 4 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | Death | | | | | | | | | | |
| 5 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 10 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 11 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 12 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 16 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 17 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 18 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 22 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 23 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 24 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

- 1= Normal
- F = Female
- 75 = Moribund Status (Being in the state of dying. A diagnosis and decision point based on appropriate clinical judgment, taking into account the severity of the condition, prognosis, potential loss of data, pain and distress).

**Gross Pathology**

Gross pathology revealed that In RPVY Set I, animals 1 and 2 were found dead with the test item present in their stomachs, which were tympanic. and all other organs appeared normal. In animal 3, the stomach was tympanic with no food present and the intestines were empty, all the remaining organs were normal in the gross necropsy, no abnormalities were found in any of the animals from Set II and III. In RPEMF, Set I, animal number 4 found dead with the test item was not present in the stomach and intestine. Feed also not present in stomach. The trachea and esophagus were intact, and all other organs appeared normal. Necropsy of the animals that survived revealed no gross pathological abnormalities in internal organs, further indicating a lack of significant toxicological effects.

**Table 6: Gross Pathology Observations**

| Sample | Dose (mg/kg) | Findings |
|---|---|---|
| RPVY | 300 | Tympanic stomach, test item present |
| | 50 | No abnormalities detected |
| RPEMF | 300 | One animal with empty stomach, others normal |
| | 2000 | No abnormalities detected |

**DISCUSSION**

The acute toxicity study of two samples of *Rasapushpa*, RPVY (prepared by the classical *Valukayantra* method) and RPEMF (prepared by the Electric Muffle Furnace method), showed differences in toxicity. The $LD_{50}$ value for RPVY was greater than 50mg/kg, classifying it under GHS Category 4, indicating moderate toxicity, while RPEMF had an $LD_{50}$ greater than 2000mg/kg, categorizing it under GHS Category 5 or Unclassified, suggesting low toxicity. In the gross pathology, two animals in Set I of RPVY were

found dead with the test substance in their tympanic stomachs, and a third animal had an empty stomach and intestines. No abnormalities were observed in other animals. In RPEMF, one animal in Set I was found dead without the test substance or feed in the stomach, and all organs appeared normal. The difference in toxicity could be attributed to factors like particle size and surface area; RPVY's smaller particle size (263µm) increases its surface area, enhancing reactivity, absorption, and toxicity compared to RPEMF (628µm), which has larger particles. Additionally, smaller particles may dissolve more readily in the body, leading to higher toxicity. A comparison with calomel pharmacokinetics shows that, like calomel, RPVY is poorly absorbed, with minimal metabolism, while RPEMF may have different absorption dynamics.

Human equivalent doses were calculated using the CSIR dose calculator. For RPVY, with an $LD_{50}$ greater than 50mg/kg, the equivalent human dose for a 70kg person is approximately 5.5g. For RPEMF, with an $LD_{50}$ greater than 2000mg/kg, the equivalent human dose is approximately 22.7g. In comparison, the therapeutic dose of *Rasapushpa*, as reported in Rasatarangini, is between 62.5 to 312.5 mg (0.5 to 2.5 *Gunja*),[6] well below the calculated human $LD_{50}$ doses. Therefore, both formulations are considered safe within the classical dosage limits, with RPEMF demonstrating a safer profile than RPVY.

## CONCLUSION

This study established the acute toxicity profile of *Rasapushpa*, comparing two different preparation methods: the classical *Valukayantra* method and the Electric Muffle Furnace method. Both RPVY and RPEMF are considered safe within classical dosage limits, with therapeutic doses significantly lower than their calculated human $LD_{50}$ values. RPVY's equivalent human dose is 5.5 g, while RPEMF's is 22.7gm. Compared to these, the therapeutic dose of *Rasapushpa* (62.5–312.5mg) is much lower concluding it is a safe mercurial preparation for various therapeutic modalities into a given classical dose.

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