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# ACUTE ORAL TOXICITY STUDY OF HRIDAYARNAVA RASA PREPARED APPLYING UPADHATU SIDDHANT OF AYURVED PRAKASH ON SPRAGUE DAWLEY RATS

[1]Dr Rakesh Salve [2]Dr Rohini Salve [3]Dr M R Pandya
[1]PhD. Scholar, Dept. of RS & BK [2]PhD. Scholar, Dept. of Panchkarma [3]Retd. Professor
Dept. of RS & BK, Parul Institute of Ayurved, Vadodara, Gujarat.

## ABSTRACT

Cardiovascular diseases (CVDs) were responsible for 20.5 million deaths in 2021, comprising about one-third of all global deaths, a sharp rise from the 12.1 million deaths recorded in 1990. Hridayarnava Rasa is a herbo-mineral compound which is used in all types of Cardiovasular diseases. Upadhatu Siddhant enlists names of representatives of metals (Upadhatu) which can replace original metals to exhibit similar action. The present study aimed to carry out toxicity study of modified Hridayarnava Rasa (HR-2) when administered as a single dose to rats, followed by an observation period of 14 days. HR-2 is a novel herbo-mineral formulation where Tamra Bhasma is replaced by Tuttha Bhasma. The acute toxicity study of HR-2 was conducted as per the OECD Guideline for the testing of Acute Toxic Class Method No.423. No mortality was observed in all treated rats at the dose level of 2000mg/kg. Based on the results, single oral administration of HR-2 in female Wistar rats at a dose level 2000 mg/kg did not result in any mortality under the conditions and procedures followed in the study. We can conclude that HR-2 is safe and might be a potential management option for Cardiovasular Diseases especially related to dyslipidaemia related complications.

**KEYWORDS:** Ayurved, Experimental, Dyslipidaemia, Hridayarnava Rasa

## INTRODUCTION

Global trends in cardiovascular disease (CVD) burden are shifting due to complex interactions between exposures to modifiable risk factors, aging populations, and changing access to healthcare. This information on the drivers of change in CVD burden can help in setting priorities for public health policies, developing targeted prevention strategies, and identifying effective treatment strategies. This information will also help in the achieving the Sustainable Development Goals Target 3.4, which calls for a reduction of premature mortality from non-communicable diseases by one-third by the year 2030[1]. The leading cause of death in 1990 in India was from diarrheal diseases, while in 2019, most deaths were due to ischemic heart disease followed by chronic obstructive pulmonary disease[2]. The all-age prevalence of most leading NCDs increased substantially in India from 1990 to 2016, but the age standardised prevalence increased only for diabetes, cerebrovascular disease, ischaemic heart disease, and skin diseases[3]. Dyslipidaemias are raised total, LDL and non-HDL cholesterol, triglycerides and lipoprotein(a) and low HDL cholesterol.[4]Dyslipidemia is one of the most important risk factors for atherosclerotic cardiovascular disease (ASCVD), which accounts for the most deaths worldwide.

Maintaining a healthy level of blood cholesterol is an important prevention strategy for ASCVD, through lifestyle intervention or cholesterol-lowering therapy[5].

Hridayarnava Rasa can be considered as one of such cholesterol lowering therapy which is widely indicated and used by Ayurved physicians in their day-to-day practice. It is a herbo-mineral formulation which is a combination of processed Mercury (Shuddha Parada), processed Sulphur (Shuddha Gandhak) and processed Copper (Tamra Bhasma) which is triturated with the decoction of Triphala (3 myrobalans) and juice of Kakamachi (Solanum nigrum)[6]. Tuttha has been indicated by Ayurved Prakash as the upadhatu of Tamra[7] which is similar in its pharmacological actions to Tamra and can be used as its representative in place of Tamra Bhasma. Though Tamra is abundantly available, the process of Tamra Marana is time consuming as compared to its upadhatu i.e., Tuttha. Tuttha is also readily available and its process of incineration or marana comparatively takes less time and yields Tuttha Bhasma in less labour. Tuttha Bhasma was added to Hridayarnava Rasa as a substitute to Tamra Bhasma in Hridayarnava Rasa, keeping the rest of the contents and method of preparation undisturbed. Ample information is available as far as pharmaceutical, pharmacological, analytical as well as clinical data of Hridayarnava Rasa is concerned. However, being a novel preparation, modified Hridayarnava Rasa (HR-2) needs to go through this sequence of standardization. After proper preparation, this modified Hridayarnava Rasa was subjected to acute toxicity study in order to generate safety data which is the need of the hour, if this drug is to be further evaluated for clinical studies.

## MATERIALS AND METHODS

The raw drugs were procured from local vendors at Vadodara and Ahmedabad. Identification and authentication of raw drugs was done by Raw Drug Identification Committee (RDIC) of Parul Institute of Ayurveda, Vadodara and modified Hridayarnava Rasa (HR-2) was prepared at GMP certified Parul Ayurved Pharmacy, Vadodara.

**Table 1: Ingredients of Modified Hridayarnava Rasa (HR-2)**

| Sr. No. | Drug Name | English / Latin Name | Part Used | Quantity |
|---|---|---|---|---|
| 1 | Shuddha Parada | Mercury | Processed | 1 part |
| 2 | Shuddha Gandhak | Sulphur | Processed | 1 part |
| 3 | Tuttha Bhasma | Copper Sulphate | Processed | 1 part |
| 4 | Triphala Kwatha | 3 Myrobalans | Fruit Decoction | Q.S. |
| 5 | Kakamachi Swaras | *Solanum nigrum* | Leaf juice | Q.S. |

**Preparation of HR-2:** Preparation of modified Hridayarnava Rasa was carried out at GMP certified Pharmacy of Parul Institute of Ayurved, Vadodara, Gujarat. Parada was obtained by Hingulottha[8] method which is considered as the purest form and is indicated to be used in medicines without the need of any further processing. Gandhak was subjected to shodhan[9] to remove physical impurities as well as incorporate properties like rasayana. Tuttha was converted into Bhasma form with the help of shodhan and marana process[10,11], which was used as a substitute for Tamra Bhasma. Freshly prepared decoction of Triphala and juice of Kakamachi were used for the process of bhavana. On proper drying, this HR-2 sample was analysed for organoleptic and physico chemical parameters. The safety profile of HR-2 was evaluated by conducting acute toxicity study as per OECD guidelines.

**Study Guidelines:**

The study was intended to provide information for LD50 cut-off values for the

classification of chemical substances. Data from the study will serve a basis to establish a ranking of a substance to be classified for the purpose of hazard assessment. The design and scope of the study is based on consideration of the study objectives. The experimental procedures were performed based on standards set forth in OECD guideline for testing of chemicals, No. 423, 'Acute Oral toxicity - Acute Toxic Class Method', adopted on 17th December 2001 (OECD, 2001). The experimental procedures were performed based on protocol set forth and approved by IAEC Committee.

**Study Duration and Protocol Number:**

The study was performed in September 2024 (02.09.24 to 04.10.24) and approved Project Proposal No.: SC/IAEC/2024/037.

**Experimental Procedure Justification of Selection System:**

The Sprague Dawley Rats *(Rattus norvegicus)* were selected as the test system because it is a readily available rodent species. Rats were selected because there is a large volume of background data on this species. Specified in OECD Guidelines for Testing of Chemicals, 423 standards, as an appropriate test to evaluate the oral toxicity of test item and recommended by various regulatory authorities. The test system was approved by Sciore Research Private Limited's Institutional Animal Ethics Committee (IAEC).

**Table 2: Test system details:**

| | |
|---|---|
| Species | Sprague Dawley Rats *(Rattus norvegicus)* |
| Sex | Female (nulliparous and non-pregnant) |
| Age | 6 to 8 weeks |
| Body weight range | 100-130 gm |
| No. of animals | Three [3] animal /step (total 6 animal) |
| Source | Sciore Research Private Limited |
| Accommodation | 3 Female rats were housed in a single stainless-steel cage with facilities for food and water bottle. |
| Diet | Standard Pelleted feed (Maintenance Diet) |
| Water | RO Water |
| Dose | 2000 mg/kg body weight (Limit test dose for toxicity study as per the guidelines of OECD 423 |
| IAEC Project Proposal No. | SC/IAEC/2024/037 |

**Table 3: Experimental Design for acute toxicity of HR-2**

| Step | Dose (mg/kg) | Number of animals | Number of moribund or dead animals | Subsequent action |
|---|---|---|---|---|
| 1 | 2000 | 3 females | 0 to 1 | Proceed to Step 2 |
| | | | 2 to 3 | Proceed to Step 3 (300 mg/kg dose) |
| 2 | 2000 | 3 females | 0 to 1 | Classification of substance as per GHS |
| | | | 2 to 3 | Proceed to Step 3 (300 mg/kg dose) |

**Housing of Animal:**

Animals were housed in groups (3 animal per cage) in clean, sterilized poly-carbonate cages having provision for holding pelleted food and drinking water in bottle with stainless steel nozzle throughout the study period. The quantity and quality of feed and water were regularly monitored. Cages and water bottles were cleaned as per the Standard Operating Procedure (SOP) of Good Laboratory Practice (GLP) (17).

**Environmental Conditions:**

- Temperature: 21+3ºC
- Relative humidity: 50±5%
- Light/dark cycle (photoperiod): 12-hour light and 12-hour dark cycle

**Experimental Procedure:**

The test product was administered by an oral route to each animal by a single oral gavage as shown in following table. All the animals were fasted for approximately 15 to 16 hours before administration of test item, but with free access to water. The animals were dosed using a stainless-steel intubation needle fitted onto a suitably graduated syringe. The dose administration was conducted in step wise manner as indicated in OECD Guideline 423 (OECD, 2001) and SOP: SC/TOX/SOP/001. Food was supplied approximately 3h to 4h after test item administration.

**Table 4: Dose administration of modified Hridayarnava Rasa (HR-2)**

| | Animal ID | Body weight (gm) | Volume administered (ml) |
|---|---|---|---|
| **Step 1 (200 mg/ml)** | H | 100.00 | 1.00 |
| | B | 105.00 | 1.05 |
| | T | 111.00 | 1.11 |
| **Step 2 (200 mg/ml)** | HB | 116.00 | 1.16 |
| | BT | 121.00 | 1.21 |
| | HT | 132.00 | 1.32 |

**Observations:**

Mortality and Morbidity: All animals were observed for mortality and morbidity for a period of 14 days after administration of test item. If no morality or morbidity is observed at particular step, the next step was started.

**Body Weight Recording:** Body weights of each animal were recorded prior to test item administration (Day 0) and on Day 7 and Day 14 of step.

**Clinical Observations:** Clinical observations were performed to look for signs of ill health or over toxicity during the first 30 min and at approximately 1, 2, 3, and 4h after administration of test item and then everyday till the completion of experiment step. Any appearance of abnormalities, behavioural changes or other signs of reaction to treatment or ill health were recorded and detailed individual record was maintained.

**Gross Pathological Examination:** All the survived animals were subjected to gross necropsy at the end of observation period and subjected to gross pathology.

**Evaluation Criteria:** According to the "Globally Harmonized System (GHS) for classification of chemicals which cause acute toxicity, OECD series on testing and assessment, Number 33; Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemicals Substances and Mixtures [ENV/JM/MONO (2001)6]".

**Table 5: GHS classification system of chemicals**

| LD50 | GHS Category |
|---|---|
| >0-5 | Category 1 |
| >5-50 | Category 2 |
| >50-300 | Category 3 |
| >300-2000 | Category 4 |
| >2000-5000 | Category 5 |

## RESULTS

Changes in Body weight: Body weights of each animal were recorded prior to the test item administration (Day 0) and on Day 7 and Day 14 of the experiment. Body weights changes are indicated in the following tables.

Mortality and Clinical Signs Observation: All the animals were observed for mortality and morbidity for a period of 14 days following the test item administration. No mortality or morbidity was observed at a particular experimental step 1. As there was no mortality or morbidity observed at step 1, step 2 was initiated at Day 7.

Clinical Observations: During the study animals were evaluated for cage side observations and clinical observations. The changes in clinical observations are indicated in terms of AG: Abnormal Gait; AP: Abnormal Posture; AI: Any other signs of illness; HL: Hair Loss; LC: Lacrimation; LM: Lameness; LT: Lethargy; ND: Nasal discharge; PD: Physical deformities; SL: Salivation; TR: Tremors/convulsions; UR: Urination; NC: No clinical changes observed; and D: Dead. The summary of clinical observation is given in following tables.

Gross Necropsy Observations: At the end of study animals were sacrificed and gross necropsy was conducted. Observations from gross necropsy are given below.

Evaluation of Results: During the study no animals were found dead. Therefore, as per GHS classification based upon LD50 mentioned in OECD 423, the Test Item were classified under GHS category 5 with cut off LD 50 at 5000 mg/kg.

**Table 6: Body Weight Measurement (gm):** Step 1

| Animal Marking | **Day 0** | **Day 7** | **Day 14** |
|---|---|---|---|
| H | 100.00 | 112.00 | 126.00 |
| B | 105.00 | 118.00 | 130.00 |
| T | 111.00 | 125.00 | 140.00 |
| Mean | 102.33 | 118.33 | 132.00 |
| SD | 5.51 | 6.51 | 7.21 |

**Table 7: Difference in Body Weight Measurement (gm):** Step 1

| Animal Marking | **Day 7** | **Day 14** |
|---|---|---|
| H | 12.00 | 26.00 |
| B | 13.00 | 25.00 |
| T | 14.00 | 29.00 |
| Mean | 13.00 | 26.67 |
| SD | 1.00 | 2.08 |

**Table 8: Body Weight Measurement (gm):** Step 2

| Animal Marking | **Day 0** | **Day 7** | **Day 14** |
|---|---|---|---|
| HB | 116.00 | 130.00 | 147.00 |
| BT | 121.00 | 135.00 | 148.00 |
| HT | 123.00 | 136.00 | 152.00 |
| Mean | 120.33 | 133.67 | 149.00 |
| SD | 3.61 | 3.21 | 2.65 |

**Table 9: Difference in Body Weight Measurement (gm):** Step 2

| Animal Marking | **Day 7** | **Day 14** |
|---|---|---|
| HB | 14.00 | 31.00 |
| BT | 14.00 | 27.00 |
| HT | 13.00 | 29.00 |
| Mean | 13.67 | 29.00 |
| SD | 0.58 | 2.00 |

**Table 10: Clinical Observations:** Step 1

| Markings | Hrs | | | | | Days | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | ½ | 1 | 2 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 12 | 14 |
| H | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC |
| B | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC |
| T | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC |

*NC – No clinical changes

**Table 11: Clinical Observations:** Step 2

| Markings | Hrs | | | | | Days | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | ½ | 1 | 2 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 12 | 14 |
| HB | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC |
| BT | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HT | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC | NC |

*NC – No clinical changes

**Table 12: Gross Necropsy Observations:** Step 1

| **Animal ID** | **H** | **B** | **T** |
|---|---|---|---|
| External Organs: Hairs, Skin, Scars, Lesions, Eyes, Ears, Mouth, Nares, Orifices, Mucous Membranes | NAD | NAD | NAD |
| Skin level Incision: Subcutaneous fat, Musculature, Superficial Lymph nodes | NAD | NAD | NAD |
| Endocrine glands: Thyroid, parathyroid, Adrenal, Pitutiary | NAD | NAD | NAD |
| Thoracic cavity: Lungs, Heart, Thymus, Trachea | NAD | NAD | NAD |
| Peritoneal cavity: Liver, Pancrea, Spleen, Kidney, Adrenals, Urinary bladder | NAD | NAD | NAD |
| Gastrointestinal Tract: Oesophagous, Stoamch, Small intestine, large intestine, rectum | NAD | NAD | NAD |
| Genitals: Ovaries, Uterus, Vagina | NAD | NAD | NAD |
| Bones and Joints | NAD | NAD | NAD |
| Brain and Spinal Cord | NAD | NAD | NAD |
| Summary | -- | -- | -- |

**Table 13: Gross Necropsy Observations:** Step 2

| **Animal ID** | **HB** | **BT** | **HT** |
|---|---|---|---|
| External Organs: Hairs, Skin, Scars, Lesions, Eyes, Ears, Mouth, Nares, Orifices, Mucous Membranes | NAD | NAD | NAD |
| Skin level Incision: Subcutaneous fat, Musculature, Superficial Lymph nodes | NAD | NAD | NAD |
| Endocrine glands: Thyroid, parathyroid, Adrenal, Pituitary | NAD | NAD | NAD |
| Thoracic cavity: Lungs, Heart, Thymus, Trachea | NAD | NAD | NAD |
| Peritoneal cavity: Liver, Pancreas, Spleen, Kidney, Adrenals, Urinary bladder | NAD | NAD | NAD |
| Gastrointestinal Tract: Oesophagus, Stomach, Small intestine, large intestine, rectum | NAD | NAD | NAD |
| Genitals: Ovaries, Uterus, Vagina | NAD | NAD | NAD |
| Bones and Joints | NAD | NAD | NAD |
| Brain and Spinal Cord | NAD | NAD | NAD |
| Summary | -- | -- | -- |

## DISCUSSION

The above results show that single oral administration of modified Hridayarnava Rasa (HR-2) in female Sprague Dawley Rats *(Rattus norvegicus)* at a dose level 2000 mg/kg b.wt. did not result in any mortality under the conditions and procedures followed in the study. Hence the LD50 cut off value for the test item i.e modified Hridayarnava Rasa (HR-2) was 5000 mg/kg b.wt. or infinitive. Additionally, considering globally harmonized system (GHS) for the classification of chemicals, the test item was classified under category 5 or unclassified. The present study aimed to evaluate the acute oral toxicity of modified Hridayarnava Rasa (HR-2), in search of formulate a novel herbal formulation designed for managing Dyslipidaemia which is considered as a prime participant in Cardiovascular Disorders (CVD). The results indicated that modified Hridayarnava Rasa (HR-2) was

well tolerated at the highest dose of 2000 mg/kg body weight, with no observed mortality or morbidity in the tested animals. Clinical signs, including skin and fur conditions and behavioural patterns, remained normal throughout the study period. Additionally, body weight gain was observed in all animals, suggesting no adverse impact on general health. The absence of toxic effects in acute oral toxicity testing supports the safety of modified Hridayarnava Rasa (HR-2) for further pharmacological evaluation. The gross necropsy observations like external organs, skin level incisions, endocrine glands, thoracic activity, peritoneal cavity, gastrointestinal tract, genitals, bones and joints as well as brain and spinal cord did not show any abnormalities suggesting of no adverse systemic impact. These observations imply that the formulation does not cause significant systemic toxicity. However, chronic toxicity studies, along with detailed pharmacodynamic and pharmacokinetic evaluations, are necessary to establish long-term safety and efficacy. Future research should also focus on clinical trials to assess the effectiveness of this novel formulation in human subjects. The study underscores the potential of traditional herbal medicine in addressing global health challenges such as dyslipidaemia induced cardiovascular diseases, emphasizing the need for integrative approaches in modern healthcare.

## CONCLUSION

In this study, on the basis of observations of the mortality-morbidity rate, clinical signs, body weight and gross pathology, it can be concluded that the novel modified herbo-mineral formulation Hridayarnava Rasa is safe and it might be a potential management option for dyslipidaemia induced cardiovascular diseases.

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**CORRESPONDING AUTHOR**
Dr Rakesh Salve
PhD. Scholar, Dept. of RS & BK
Parul Institute of Ayurved, Vadodara, Gujarat-India
E-mail: rakeshsalve83@gmail.com

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