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ORA- Experimental Study

Acute Oral Toxicity Evaluation of Vangadi Yoga, a Classical Herbo-Metallic Formulation in Wistar Albino Rats Conducted According to OECD Test Guideline 423

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ABSTRACT:

Background: *Vanga Bhasma*, is known to be effective in disorders related to *Shukra* is used either singularly or with multiple ingredients. Among them, *Vangadi Yoga* containing *Vanga Bhasma*, *Shilajitu*, *Apamarga Panchanga Churna,* processed with *Shalmali Moola Twak Kwatha,* was tested for its acute toxicity in Wistar Albino rats. **Aims:** The article focuses on acute toxic effects of *Vangadi Yoga* as per OECD test guideline 423. **Methods and Materials:** Acute toxicity study was carried out in accordance with OECD Test Guideline 423 using nine healthy adult male Wistar Albino rats (8-10 weeks old) divided into three experimental groups (n = 3 rats/group). *Vangadi Yoga* was given orally as a single dose in two groups of animals using the sequential dose levels of 300 mg/kg (intermediate dose) and 2000 mg/kg (limit test dose) dissolved in 0.5% CMC solution. In vehicle control group, only 0.5% CMC solution was administered. **Results:** After 14 days of observation, no mortality or major signs of clinical toxicity were found following administration of *Vangadi Yoga* at either 300 mg/kg or 2000 mg/kg. Body weight increased normally throughout the study. Gross pathological examination revealed no treatment-related abnormalities. However, few histopathological changes were observed, including focal tubular necrosis in the kidney, focal gliosis in the brain, mucosal gland hypertrophy in the stomach, and coronary congestion without ischemic changes in a few animals at limit dose. **Conclusion:** *Vangadi Yoga* demonstrated low acute oral toxicity, with LD50 value greater than 2000 mg/kg body weight according to OECD Test Guideline 423. Although no mortality or severe clinical toxicity was observed, minimal-to-mild microscopic lesions were identified in selected organs at the limit dose. These findings indicate a preliminary favorable acute safety profile, while highlighting the need for repeated-dose toxicity studies incorporating biochemical and hematological assessments to comprehensively establish the safety of the formulation.

KEYWORDS: Acute toxicity, Bhasma, Histopathology, OECD test guideline 423, Vangadi Yoga

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1. INTRODUCTION

Vangadi Yoga is a classical herbo-mineral formulation mentioned in Rasa Tarangini, [1] which is particularly indicated for disorders related to male infertility. *Vangadi Yoga* consists of *Vanga Bhasma*, *Shilajitu*, *Apamarga Panchanga Churna* processed in *Shalmali Moola Twak Kwatha.* Among all the ingredients *Vanga Bhasma* is the principal component. Experimental studies show that *Vanga Bhasma* has direct effect on the quality of Semen, [2] when used within therapeutic limits. Another important ingredient *Shilajitu*, is known for its *Rasayana* properties, which can be attributed to fulvic acid and other antioxidants present in the drug. [3] *Apamarga* has documented antioxidant and anti-inflammatory properties that also may contribute to enhance the effect of the formulation. The formulation is processed in *Kwatha* of *Shalmali* root bark which is expected to also enhance the therapeutic effects of the formulation. Even though this formulation is traditionally accepted, the scientific data regarding the acute toxicity profile is not available in the published literature. This gap in the evidence is important to be validated as it contains a metallic ingredient in the form of *Vanga Bhasma*. Although the pharmaceutical processing followed for preparation of *Bhasma* in *Rasashastra*, is intended to transform the raw metals into biologically safer forms, current scientific framework require objective safety evaluation through toxicological studies. As acute oral toxicity studies represent first step, in assessment of safety and provides information regarding the clinical toxicity signs, changes in behavior and alterations in body weight, etc., it is an important step in establishing safe dose range of the drug. Although the individual ingredients of *Vangadi Yoga*, including *Vanga Bhama, Shuddha Shilajatu, Apamarga, and Shalmali* have been investigated individually for their pharmacological and toxicological properties, safety profile of complete classical herbo-metallic formulation viz., *Vangadi Yoga* has not been scientifically established. Classical Ayurvedic pharmaceutical processing i.e. *Shodhana*, *Jarana*. *Marana*, *Bhavana*, and a combination of multiple ingredients may alter the physicochemical characteristics, bioavailability, and toxicological behavior of the final formulation. So, evaluation of the final formulation was necessary, as safety data of individual ingredients cannot be directly applied to the combined formulation. Therefore, the present study was designed to evaluate the acute oral toxicity profile of *Vangadi Yoga* in male Wistar albino rats according to the OECD Test Guideline 423 at sequential dose levels of 300mg/kg and 2000mg/kg body weight. The dose of 300 mg/kg represents an intermediate dose level, whereas 2000 mg/kg is considered a limit dose.

Aim & Objectives: To evaluate the Acute toxicity of *Vangadi Yoga* in Wistar Albino rats according to OECD Test Guideline 423.

2. MATERIALS & METHODS

Ethical Clearance: The study protocol was prior submitted, and approval was obtained from the Institutional Animal Ethics Committee (IAEC) of the institute (Approval no. PIPR 984/2025/01/17, dated 30/08/2025). The committee operated under the authorization of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Every aspect of the study, like housing conditions, dosing procedures, and welfare monitoring, was conducted as per the CPCSEA guidelines. [4] A detailed study protocol was prepared before initiation of the study and reviewed during the IAEC approval process. However, the protocol was not prospectively registered in a public repository.

Collection of Raw Drugs: The raw material, viz. *Ashuddha Vanga*, *Ashuddha Shilajatu, and Apamarga Panchanga Churna* were procured from authentic sources, while *Shalmali Moola Twaka* was procured from peripheral areas of Gujarat. All the raw drugs were authenticated from the Department of Rasa Shastra & Bhaishajya Kalpana and the Department of Dravyaguna in the institute.

Preparation of *Vangadi Yoga*: *Vangadi Yoga* with human dose of 500 mg as daily dose is a herbo-metallic formulation that consists of *Vanga Bhasma* (125 mg),

Shuddha Shilajatu (125 mg), *Apamarga Panchanga Churna* (250 mg) processed in *Shalmali Moola Twaka Kwatha*. Raw *Vanga* was processed by performing its *Shodhana,* [5] *Jarana,* [6] & *Marana* [7] and was converted into *Bhasma* form. Total 11 *Putas* at 600 °C in EMF were given to convert it into *Bhasma* form. *Shodhana* of raw *Shilajatu* was done in *Triphala Kwatha,* [8] *Vanga Bhasma, Shuddha Shilajatu and Apamarga Panchanga Churna* were sieved from 80 mesh sieve and mixed respectively in a mortar and pestle, and one *Bhawana* of *Shalmali Moola Twaka Kwatha* was given. The prepared *Vangadi Yoga* was dried, sieved, and stored in an air-tight container. It was then tested for its quality control analysis (viz. pH, Loss on Drying, Ash Value, HPTLC, XRD, XRF, FTIR, ICPOES, etc), which showed all the parameters within permissible limits. Whereas *Vanga Bhasma* passed all the classical signs of *Bhasma Pariksha* like *Varitaratva, Nishchandratva, Rekhapurnatva*, etc. [9]

Acute Toxicity Study:

Experimental Animals: Healthy adult male Albino Wistar rats were procured from Global Bioresearch Solutions Private Limited, India. Animals were acclimatized for a period of 14 days prior to initiation of the experiment. These animals were certified to be healthy and free from disease. Only male Albino Wistar rats were preferred for acute toxicity studies as the formulation was expected to show spermatogenic effect in further study. The individual rat was considered the experimental unit for all observations and analyses performed during the study. Rats were housed in polypropylene cages with stainless steel mesh tops, with three animals per cage, under standard laboratory conditions maintained at a temperature of 22 ± 2 °C, relative humidity of 50–60%, and a 12-hour light/dark cycle. Throughout the study period, corn cob bedding was provided and changed on a regular basis to ensure hygienic conditions. The animals were fed a standard laboratory pellet diet and unlimited access to drinking water ad libitum was provided. Animals were observed daily throughout the acclimatization and experimental period to monitor their health. Every experiment was carried out in accordance with the rules set forth by the Government of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee (IAEC) examined and approved the experimental protocol.

Inclusion and Exclusion Criteria: Healthy Albino Wistar male rats, eight to ten weeks of age, weighing between 200 g and 250 g, were included in the study. Animals with any signs of illness, injury, abnormal behavior, or significant weight variation during acclimatization were excluded.

Dose Selection: The classical therapeutic human dose of *Vangadi Yoga* is 500 mg/day. For dose translation from humans to experimental animals, the body surface area (BSA) conversion method described by Reagan-Shaw et al. was used which is the standard approach for converting a human dose to an animal-equivalent dose (AED) and is based on species-specific Km factors (a constant relating body weight to body surface area: Km = 37 for a 60 kg human, Km = 6 for a rat). For a reference adult body weight of 60 kg, the human dose corresponds to 500/60 = 8.33 mg/kg body weight. Using the relationship AED (mg/kg) = Human dose (mg/kg) × (Km human ÷ Km rat) = 8.33 × (37 ÷ 6), the calculated rat-equivalent dose of the human therapeutic exposure is approximately 51.4 mg/kg body weight. [10]

However, in the present acute toxicity study, the therapeutic equivalent dose was not evaluated because the objective was hazard identification according to OECD Test Guideline 423 (Acute Toxic Class Method). Therefore, a dose of 300 mg/kg body weight (approximately six times the therapeutic equivalent rat dose) was selected as the intermediate dose, while 2000 mg/kg body weight (approximately 39 times) was selected as the OECD recommended limit dose to assess acute oral toxicity and classify the formulation's toxicity potential under the Globally Harmonized System (GHS). [11]

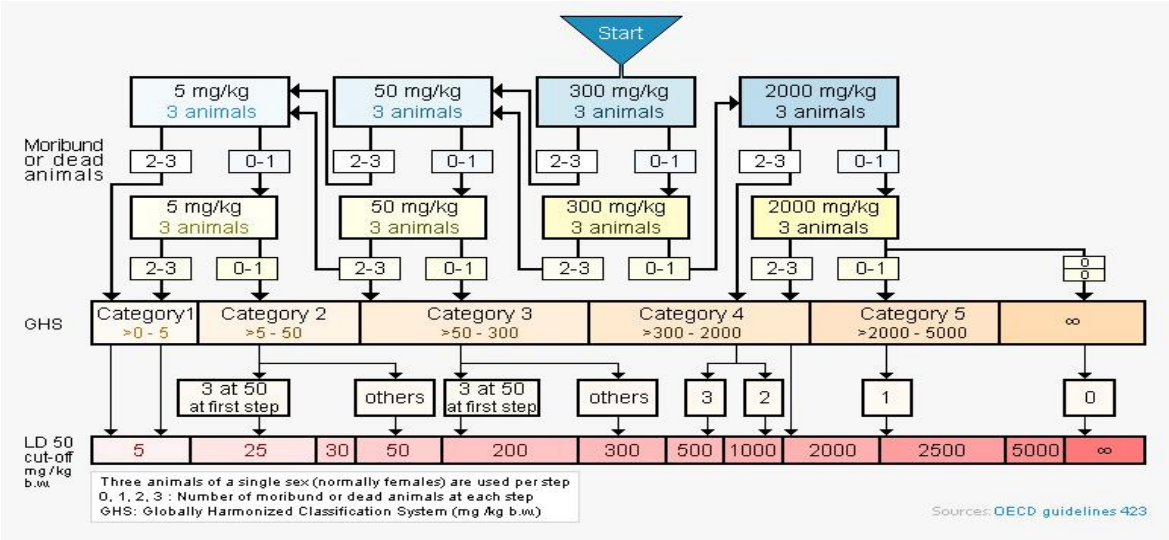

Fig 1: Flowchart of the Acute Toxic Class Method described in OECD Test Guideline 423, illustrating the sequential dose progression, mortality-based decision criteria, and corresponding Globally Harmonized System (GHS) toxicity classification categories.

Dose preparation and administration: The test formulation was suspended in 0.5% Carboxymethylcellulose (CMC) in distilled water. The dosing solutions were prepared fresh on the day of administration to ensure stability and uniformity. Dose concentrations were adjusted to ensure a constant dose of 10 ml/kg body weight for all dosage groups, as recommended under OECD Test Guideline 423. Administration was performed by oral gavage using a rounded-end stainless steel intragastric cannula connected to a disposable syringe, following an overnight fast. Body weight was measured on the morning of dosing, and individual doses were calculated. **Groups of experimental Animals** (Table 1)

Table 1: Groups of Wistar Albino rats for Acute Oral Toxicity Study

Group	DOSE	BODY WEIGHT (g)	VEHICLE
Group 1	Vehicle Control	220±10	0.5% CMC
Group 2	300 mg/kg	235±10	0.5% CMC
Group 3	2000 mg/kg	230±10	0.5% CMC

The Organization for Economic Co-operation and Development (OECD) Test Guideline 423 (Acute Toxic Class Method) was followed in conducting the acute oral toxicity investigation [11]. Before the test drug was administered, the animals were kept nil by mouth for solid foods for around 6 hours, during which time they had free access to drinking water. Three hours after drug administration, the animals were allowed to resume eating. The sample size of the animals was selected according to OECD TG 423 (Acute Toxic Class Method), which recommends use of three animals per sequential step for acute toxicity hazard classification. As OECD Test Guideline 423 is a regulatory hazard-classification guideline rather than a hypothesis-testing study, so no formal sample-size calculation or statistical power was performed. As per the sequential study prescribed by OECD TG 423, formal randomization was not performed. To minimize allocation bias, healthy animals of comparable age and body weight were selected after the acclimatization period and assigned sequentially to the study groups. The study followed a sequential dosing design with two dose levels, viz., 300mg/kg and 2000 mg/kg body weight. Each dose group contained 3 male rats, with an additional group of 3 rats kept in a vehicle control group. The study was conducted in a stepwise manner; the Intermediate dose level 300 mg/kg group was dosed first, followed by the limit dose 2000 mg/kg dose. Both groups received a single dose and a 14-day observation period. In compliance with OECD guidelines, clinical observations included monitoring for mortality, behavioral abnormalities, effects on the autonomic and central

nervous systems, and general indications of toxicity were done.

Outcome Measures: The primary outcome measure was to check for mortality after administration of the test drug. Secondary outcome measures included clinical signs of toxicity, behavioral alterations, body weight changes, gross pathological findings, and histopathological changes in major organs.

Clinical and behavioral observations: Animals were observed individually for clinical signs and behavioral changes at an interval of thirty minutes, three, six, twelve, and twenty-four hours after dosing on day one, then twelve hourly on day two & three, and once daily thereafter for day four to fourteen. Throughout the trial period, every animal was checked twice a day for illness and mortality. To identify any sign of toxicity, observations were recorded, including general appearance, skin and fur condition, eyes, respiration, autonomic and central nervous system impacts, and behavioral changes. Body weight of all the animals was measured on day one (before dosing), seven, and fourteenth day. Daily food and water intake were monitored as a routine animal welfare assessment throughout the study, but quantitative daily food and water intake were not tabulated in the study. Specific signs that were monitored included tremors, convulsions, salivation, diarrhea, lethargy, changes in posture, and abnormal locomotor activity. Particular attention was paid in the first four hours following dosing, as recommended under OECD TG 423. Clinical observations were recorded qualitatively based on the presence or absence of predefined clinical signs of toxicity. A numerical toxicity scoring or severity grading system was not employed in the present study.

Humane Endpoints: Predefined humane endpoint criteria were established before initiation of the study in accordance with CCSEA guidelines. Animals exhibiting severe or prolonged signs of distress, including inability to access food or water, severe respiratory distress, continuous convulsions, marked hypothermia, or loss of more than 20% of the initial body weight, were decided to be humanely euthanized before the scheduled termination of the study.

Anesthesia and Euthanasia Procedure: On day 14, the animals were anesthetized using an intraperitoneal injection of Ketamine (90 mg/kg) and Xylazine (10 mg/kg). Adequate anesthesia was confirmed by the absence of the pedal withdrawal reflex. Animals were euthanized by administering an overdose of the anesthetic in accordance with CPCSEA guidelines.

Necropsy and Gross Pathological Examination: At the end of the 14-day observation period, all surviving animals were euthanized humanely and subjected to a thorough gross necropsy. Vital organs including the brain, heart, kidney, lungs, spleen, small intestine, large intestine, pancreas, urinary bladder, liver, stomach, and testis, were examined for gross pathological changes (color, texture, presence of lesions or hemorrhage).

Blinding: In accordance with the sequential study design prescribed under OECD TG 423, all the personnel involved in dose administration, daily clinical observation, and necropsy were well aware of the treatment allocation. However, histopathological evaluation was performed by an independent pathologist who was blinded to the treatment groups to minimize bias. Data interpretation was carried out after completion of all observations.

Histopathological Examination: Following gross examination, key organs for histopathology, viz., brain, heart, kidney, liver, stomach, and testis, were carefully excised and fixed in 10% neutral buffered formalin. Fixed tissues were processed using standard histological techniques, embedded in paraffin wax, and stained with hematoxylin and eosin (H&E). [12]

Histopathological evaluation was performed under a light microscope to assess cellular architecture and detect any treatment-related pathological alterations. Representative microphotographs were captured at 40x magnification. Lesions were qualitatively characterized as minimal/mild in extent, in keeping with the four-tier severity terminology (minimal, mild, moderate, marked) recommended for

standardized histopathological reporting. Vehicle-control organs were not processed for histopathological comparison in the present study, as sectioning was restricted to the treated groups; this is acknowledged as a limitation in Section 6 and Section 7.

Statistical Analysis: The body weight data is presented as Mean ± Standard Deviation. In accordance with OECD Test Guideline 423, acute toxicity classification is based primarily on descriptive observations, including mortality, clinical signs, body weight changes, and gross pathological findings, rather than hypothesis-testing statistical comparisons. No inferential statistical analysis was therefore performed.

ARRIVE 2.0 Compliance: The design, conduct, and reporting of this animal study were carried out in accordance with the ARRIVE 2.0 (Animal Research: Reporting of In Vivo Experiments) guidelines to promote transparent and comprehensive reporting of in vivo animal research.

3. OBSERVATION AND RESULTS

Acute Oral Toxicity: The formulation *Vangadi Yoga* was tested for acute oral toxicity in male Albino Wistar rats using OECD Test Guideline 423. During the 14-day observation period, animals that were given a single oral dose of 300 mg/kg body weight showed no signs of treatment-related side effects or mortality. The animals that were given the highest dose of 2000 mg/kg body weight were also found to be healthy throughout the 14-day observation period. As no mortality in the experimental animal group was observed at a limit dose of 2000 mg/kg body weight, the LD50 cut-off value of *Vangadi Yoga* was considered to be greater than 2000 mg/kg body weight in accordance with OECD Test Guideline 423. None of the experimental animals reached the predefined humane endpoint criteria during the 14-day observation period. Therefore, no unscheduled euthanasia was required.

Body Weight (Table 2)

All the three groups, including the vehicle control, showed a comparable and physiologically normal pattern of weight gain across the 14 days, with no dose-related decrement observed.

Clinical and Behavioral Observations: (Table 3)

All animals were observed individually for any clinical signs of toxicity periodically as mentioned before, for a total period of 14 days. Animals treated at 300 mg/kg body weight did not exhibit any abnormal clinical signs at any point during the study. Animals maintained normal posture, locomotor activity, grooming behavior, and response to stimuli. At a 2000 mg/kg body weight dose, transient signs such as mild lethargy were observed in some animals during the initial hours following drug administration; however, these effects were short-lived and resolved spontaneously without progression in 3-4 hours. No other changes were observed in any animals, and all of them were active and responsive to stimuli. No signs of tremors, convulsions, salivation, diarrhea, abnormal posture, or behavioral changes were observed in surviving animals throughout the experimental period. Overall, the clinical observations indicated good tolerability of the test drug (Table 3). Every animal in every group appeared healthy and indistinguishable from the animal of vehicle control group. Skin, fur, eye appearance and mucous membrane color remained normal throughout the full observational period. No changes in food or water consumption were observed during the study period in any of the experimental groups. However, quantitative measurements were not recorded.

Table 2: Body Weight of Wistar albino rats throughout the acute toxicity study

Animal Group	Body Weight on Day of Dosing (Day 0) (g)	Body Weight on Day 7 (g)	Body Weight on Day of Sacrifice (Day 14) (g)
Group 1 - Vehicle Control	220±10	225±10	230±10
Group 2 - 300 mg/kg	235±10	245±10	250±10
Group 3 - 2000 mg/kg	230±10	230±10	235±10

Table 3: Clinical and Behavioral observations after dosing at 300mg/kg & 2000mg/kg doses

Parameters	300 mg/kg	2000 mg/kg
Skin & fur	Normal	Normal
Eyes & mucous membranes	Normal	Normal
Respiration	Normal	Normal
Circulatory system	Normal	Normal
Autonomic nervous system	Normal	Normal
Central nervous system	Normal	Normal
Behavior & activity	Normal	Normal
Tremors/convulsions	Absent	Absent
Salivation/diarrhea	Absent	Absent
Lethargy/sleep	Absent	Transient (first 3-4 hrs)

Gross Pathological Examination (Table 4)

Table 4: Gross Pathological Examination of the organs

Organs	300mg/kg	2000mg/kg
Testis	Normal	Normal
Kidney	Normal	Normal
Brain	Normal	Normal
Stomach	Normal	Normal
Liver	Normal	Normal
Heart	Normal	Normal

Gross Histopathological findings (Table 5)

Certain histopathological findings were observed in the organs of the euthanized animals (Table 5). Histopathological slides were coded and examined by an independent pathologist blinded to the treatment groups to minimize bias.

Table 5: Histopathological findings in the organs

Organ	300mg/kg (Fig 2)	2000mg/kg (Fig 3)
Testis	No significant changes.	No significant changes.
Kidney	No significant changes.	Focal tubular necrosis.
Brain	No significant changes.	Focal area of gliosis
Stomach	Mucosal gland hypertrophy.	Mucosal gland hypertrophy.
Liver	No significant changes.	No significant changes.
Heart	No significant changes.	Congested coronaries; no ischemic changes

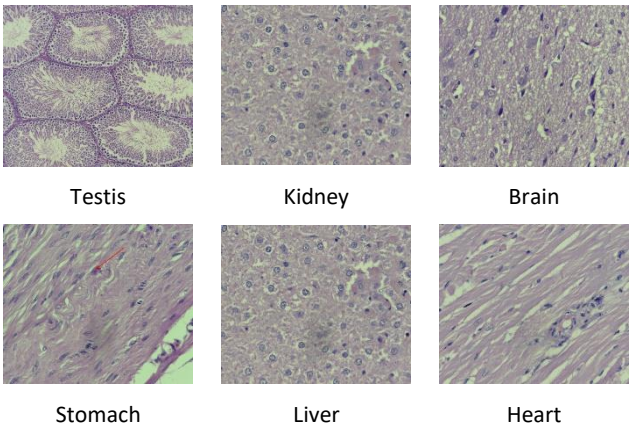

Fig 2: Microscopic images of organs of the 300mg/kg dose group at 40x.

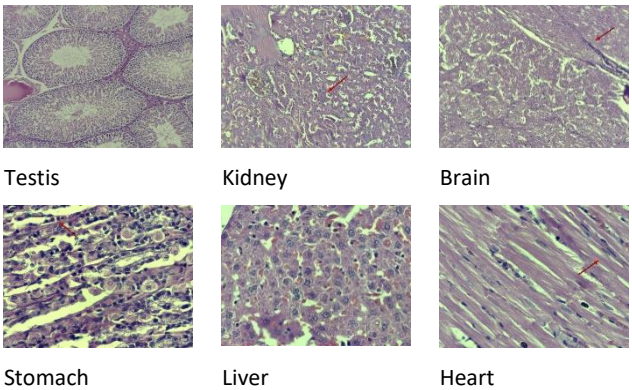

Fig 3: Microscopic images of organs of the 2000mg/kg dose group at 40x.

4. DISCUSSION

Vangadi Yoga was prepared as per classical reference, which includes *Vanga Bhasma, Shuddha Shilajitu, Apamarga Panchanga Churna* processed in *Shalmali Moola Twaka Kwatha*. All the classical signs of a good quality *Bhasma* were present in the prepared *Vanga Bhasma*.

Detailed quality control analysis that was performed also reflected all the parameters of the test drug to be within normal limits. An acute toxicity study for a span of 14 days with dosages of 300mg/kg and 2000mg/kg body weight was done as per the OECD test guideline 423. For the acute toxicity study, only Wistar Albino male rats were selected as the test drug was to be further tested for its spermatogenic effect. There was no mortality observed even at higher doses throughout the study period. Absence of severe toxic effects in the present study can be attributed to the adoption of classical processing methods for preparation of *Vanga Bhasma* and *Shuddha Shilajatu*. Procedures such as *Shodhana*, *Jarana* and *Marana* of metals and minerals are believed to transform metals/ minerals into therapeutically suitable forms that can be safely absorbed in the body. Analytical studies done on the prepared formulation also demonstrated reduced particle size (seen in SEM), and minimized the presence of free elemental metal (seen in XRD), which may be thereby help to reduce systemic toxicity in tested animals. During the study, brief episodes of mild lethargy were observed post-dosing in the 2000 mg/kg dose level group rats. This occurred within the first one to two hours following gavage size and a large suspension volume. It was resolved spontaneously within three to four hours, without any animal requiring intervention. These temporary clinical signs noted soon after administration were deemed to reflect a non-specific stress response related to gavage rather than a toxic effect from the test formulation. Oral gavage is recognized to cause short-term physiological and behavioral changes as a result of handling stress and the administration of a bolus, especially with larger doses. [13,14,15] The present study revealed that weight gain of the treated animals was normal during the time of observation, which suggests that the adverse effects of the test drug in the treated animals did not cause any adverse effect on growth, nutrient intake, or metabolic imbalance. Absence of neurological effects like tremors, convulsions, changes in locomotor activity, or sedation indicates that the formulation did not trigger any acute neurotoxicity at the given dose. Focal tubular necrosis was observed in the kidneys, focal gliosis was seen in the brain, and mild mucosal gland hypertrophy was observed in the stomach at the higher dose level, together with coronary congestion in the heart. These findings were limited, focal, and non-progressive. They were not associated with mortality, behavioral abnormalities, body weight loss or gross pathological alterations. Although the observed microscopic lesions were limited and of minimal to mild severity, their toxicological significance was interpreted cautiously because vehicle-control tissues were not subjected to histopathological examination. Consequently, direct microscopic comparison between treated and control animals was not possible, and it cannot be conclusively determined whether these lesions represent spontaneous background findings or treatment-related effects. [16, 17] Comparable patterns have been reported for other Ayurvedic herbo-metallic formulations assessed under OECD protocols. In an OECD TG 423 study of *Abhrasindoora*, a mercury-containing herbo-bio-mineral preparation dosed at the same 300 and 2000 mg/kg levels, no mortality or histopathological abnormality was observed and the formulation was placed in GHS category 5 (LD50 > 2000 mg/kg). [18] Similarly, a 28-day repeated-dose evaluation of the mercury-containing formulation *Rasasindura* demonstrated no significant histopathological or biochemical abnormalities. in brain, liver, kidney, or testis at doses up to eight times the calculated animal-equivalent therapeutic dose. While only minor, non-progressive behavioral changes were noted in a few high-dose animals. [19] It is notable, however, that a 90-day sub-chronic evaluation of the herbo-metallic formulation *Mahalaxmi Vilas Rasa* found no histopathological abnormality on light microscopy at any dose, yet detected a statistically significant increase in direct bilirubin at a moderate dose level using serum biochemistry alone. [20] This suggests that biochemical investigations can detect early organ changes that may not be visible on routine histopathology. As serum biochemistry and hematological parameters were

not evaluated in the present study, the functional significance of the microscopic changes observed in the kidney, brain, stomach, and heart at 2000 mg/kg could not be confirmed. Consequently, while the present findings are broadly consistent with the low acute oral toxicity reported for comparable herbo-metallic *Rasaushadhis*, the present dataset supports the more conservative interpretation that *Vangadi Yoga* is well tolerated based on clinical observations, body weight changes, and gross pathological examination, while its organ-level biochemical safety at the doses tested remains to be established.

Limitations of the study:

- Serum clinical biochemistry (liver function tests such as ALT, AST, ALP and bilirubin; kidney function tests such as serum creatinine, blood urea nitrogen, uric acid) and hematological parameters were not evaluated. Vehicle-control organs were also not processed for histopathological examination. Therefore, the functional and treatment-related significance of the focal lesions observed in the kidney, brain, stomach, and heart at the limit dose could not be confirmed against either biochemical data or control-tissue comparison.
- Several design constraints inherent to the OECD TG 423 protocol limits the depth of the safety assessment. The absolute and relative organ weights and quantitative food and water intake were not recorded. Clinical observations were scored qualitatively without a standardized severity grading system and no formal inferential statistical analysis was performed. The sample size of three animals per group, although was protocol-compliant, puts a limit to detect subtle or low-incidence toxicological effects.
- The study duration was limited to a single-dose, 14-day acute exposure and does not address the effects of repeated or prolonged administration. As these findings were not confirmed or excluded by biochemical or hematological data, nor compared against vehicle-control histology, the present findings should therefore not be interpreted as establishing full toxicological safety of *Vangadi Yoga*, but rather as supporting a preliminary, favorable safety signal on clinical and gross-pathological grounds. Further repeated-dose toxicity studies that incorporate clinical biochemistry, hematology, organ-weight analysis and vehicle-control histopathological comparison are warranted to confirm the safety profile of *Vangadi Yoga* before its acute safety can be considered conclusively established.

5. CONCLUSION

A single oral dose of *Vangadi Yoga* at 300 mg/kg and 2000 mg/kg body weight produced no treatment-related clinical signs beyond transient lethargy at the limit dose, with normal, comparable weight gain across all groups over the 14-day observation period. As no mortality occurred at the 2000 mg/kg limit dose, the LD50 of *Vangadi Yoga* is greater than 2000 mg/kg body weight, placing the formulation in the low-toxicity or unclassified GHS category as per OECD Test Guideline 423. At the 2000 mg/kg dose, histopathological findings showed focal tubular necrosis in the kidney, focal gliosis in the brain, mucosal gland hypertrophy in the stomach, and coronary congestion without ischemic change in the heart. The 300 mg/kg dose showed only mucosal gland hypertrophy in the stomach, with no other organ changes. These lesions were minimal-to-mild, focal, and not accompanied by mortality, clinical signs, or gross pathological change, but because serum biochemistry, hematology, and vehicle-control histopathology were not performed, their treatment-relatedness could not be confirmed. The present findings therefore support a preliminary, favorable acute safety profile for *Vangadi Yoga* under OECD TG 423. However, repeated-dose studies incorporating biochemical, hematological, and vehicle-control histopathological assessment are needed before its safety can be considered conclusively established.

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Authors Contribution:

Conceptualization and clinical management: NP

Data collection and literature search: NP

Writing – original draft preparation: NP

Reviewing & editing: MB, PM, SDM, NB

Approval of final manuscript: All authors

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement: The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Parul Institute of Ayurved & Research, Vadodara, Gujarat, India. (Approval no. PIPR 984/2025/01/17, dated 30/08/2025). All animal procedures were conducted in accordance with applicable institutional and national guidelines for the care and use of laboratory animals.

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REFERENCES:

1. Kashinath Shastri (editor). Rasa Tarangini of Sadhananda Sharma, Chapter 18, verse no.48. 11th edition, Varanasi; Motilal Banarasi Das;2021;646.
2. Chaudhary P, Lamba N, Mehra BL, Kashyap CP. Clinical evaluation of Vanga Bhasma as Vrishya. International Journal of Research in Ayurveda and Pharmacy. 2014;5(4):406-411. Available from: https://doi.org/10.7897/2277-4343.05484
3. Carrasco-Gallardo C, Guzmán L, Maccioni RB. Shilajit: a natural phytocomplex with potential procognitive activity. Int J Alzheimers Dis. 2012;2012:674142. Available from: https://doi.org/10.1155/2012/674142
4. Committee for the Purpose of Control and Supervision of Experiments on Animals. CPCSEA guidelines for laboratory animal facility. New Delhi; Ministry of Environment, Forest and Climate Change, Government of India;2023.
5. Devnath Singh Goutam (editor). Rasamritam of Yadavji Tikamji, chapter 3, verse no.87. 1st edition, Varanasi; Chaukhambha Surbharti Prakashan;2014;45.
6. Devnath Singh Goutam (editor). Rasamritam of Yadavji Tikamji, chapter 3, verse no.88-92. 1st edition, Varanasi; Chaukhambha Surbharti Prakashan;2014;45.
7. Devnath Singh Goutam (editor). Rasamritam of Yadavji Tikamji, chapter 3, verse no.93-94. 1st edition, Varanasi; Chaukhambha Surbharti Prakashan;2014;45.
8. Kashinath Shastri (editor). Rasa Tarangini of Sadhananda Sharma, chapter 22, verse no.69-78. 11th edition, New Delhi; Motilal Banarasi Das;2014;584-585.
9. Kulkarni D.A. (editor). Rasa Ratna Samucchaya of Rasa Vagbhatta, chapter 8, verse no.26-30. 1st edition, New Delhi; Meharchanda Lakshmandas Publication;2017;148.
10. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659-661. Available from: https://doi.org/10.1096/fj.07-9574LSF
11. Organisation for Economic Co-operation and Development. Test No. 423: acute oral toxicity - acute toxic class method. OECD guidelines for the testing of chemicals. Paris; OECD Publishing;2001. Available from: https://doi.org/10.1787/9789264071001-en
12. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. Cold Spring Harb Protoc. 2008;2008:pdb.prot4986. Available from: https://doi.org/10.1101/pdb.prot4986
13. Balcombe JP, Barnard ND, Sandusky C**.** Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci*. 2004;43(6):42-51. Available from: https://pubmed.ncbi.nlm.nih.gov/15669134/
14. Brown AP, Dinger N, Levine BS. Stress produced by gavage administration in the rat. Contemp Top Lab Anim Sci. 2000;39(1):17-21. Available from: https://pubmed.ncbi.nlm.nih.gov/11178310/
15. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci. 2011;50(5):600-613. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3189662/
16. Greaves P. Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation. 5th edition, London;

Academic Press;2017. Available from: https://doi.org/10.1016/C2010-0-67226-9

17. Chafer KA, Eighmy J, Fikes JD, Halpern WG, Hukkanen RR, Long GG, et al. Use of severity grades to characterize histopathologic changes. Toxicol Pathol. 2018;46(3):256-265. Available from: https://doi.org/10.1177/0192623318761348
18. Bramhankar R, Mishra AK, Das S, Munishwar N. Evaluation of acute and subacute toxicity of Abhrasindoora: as a mercury-based traditional herbo-bio-mineral metallic formulation. Pharm Sci Asia. 2023;50(3):238-246. Available from: https://doi.org/10.29090/psa.2023.03.22.187
19. Kamath SU, Rajendran K, Pemiah B, Rajan KS, Krishnaswamy S, Sethuraman S, et al. Pharmacological and toxicological insights into the ayurvedic formulation Rasasindura. Sci Rep. 2025;15(1):43591. Available from: https://doi.org/10.1038/s41598-025-97062-8
20. Patil PS, Wagh MD, Mohan M, Pande SP, Kulkarni RA, Kulkarni AN. Subchronic oral toxicity assessment of Ayurvedic herbo-metallic formulation Mahalaxmi Vilas Rasa in Wistar rats. J Drug Res Ayurvedic Sci. 2024;9(3):158-165. Available from: https://doi.org/10.4103/jdras.jdras_120_23