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Preclinical safety assessment of *Swarna Vanga* prepared by two classical ayurvedic methods: An acute oral toxicity study

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Abstract

Introduction: *Swarna Vanga*, a classical *Rasa Shastra* herbomineral formulation synthesized from tin (*Vanga*), mercury (*Parada*), and sulfur (*Gandhaka*), is widely utilized in traditional therapeutics. Despite safety concerns surrounding heavy metal precursors, classical thermal synthesis (*Kupipakwa*) is intended to transform raw elements into biologically tolerable entities. This study aimed to evaluate and compare the acute oral toxicity profiles of two textually distinct *Swarna Vanga* (SV) preparations – SV1 (Rasamrita) and SV2 (Rasatantrasara evam Siddhaprayoga Samgraha) under standardized regulatory conditions. **Materials and Methods:** The acute oral toxicity of SV1 and SV2 was evaluated in accordance with the Organisation for Economic Co-operation and Development Test Guideline 423 (Acute Toxic Class Method). Female Sprague–Dawley rats were administered single oral doses of SV1 or SV2 across starting and sequential dose levels of 5, 50, 300, and up to the limit dose of 2000 mg/kg body weight (n = 3/step). Animals were closely monitored for immediate autonomic, behavioral, and neurological changes during the first 24 h, followed by daily clinical observations and periodic body-weight tracking across a 14-day post-administration observation period. **Results:** Single-dose oral administration of either SV1 or SV2 produced 0% mortality and no signs of morbidity up to the limit dose of 2000 mg/kg. Comprehensive physiological evaluations revealed no treatment-induced alterations in autonomic, behavioral, neuromuscular, or respiratory parameters across all dose cohorts (5, 50, 300, and 2000 mg/kg). Parameters including locomotion, grooming, pupillary reflexes, salivation, and gastrointestinal output remained unperturbed throughout the 14-day observation window. Body-weight fluctuations were non-dose-dependent, non-adverse, and statistically comparable to vehicle control variations. **Conclusion:** Both *Swarna Vanga* formulations, SV1 and SV2, exhibited no evidence of significant acute oral toxicity, with no mortality or overt signs of toxicity observed at doses up to 2000 mg/kg, supporting an estimated LD_{50} >2000 mg/kg and classification within GHS Category 5/unclassified. Despite variations in classical preparation methods and ingredient ratios, no appreciable differences in acute systemic toxicity were observed, thereby establishing a preliminary safety baseline for these traditional formulations.

Key words: Herbal-mineral, OECD, preclinical, Rasa Shastra, *Swarna Vanga*, toxicity

INTRODUCTION

Traditional Ayurvedic medicine, particularly the herbo-metallic discipline of *Rasa Shastra*, utilizes intricate processing methodologies – such as *Shodhana* (purification), *Marana* (calcination), *Jarana* (roasting), and *Kupipakwa* (graduated thermal sublimation) – to transform crude metals into biocompatible, therapeutically potent compounds.[1] When administered according to strict classical protocols governing posology (*Matra*), delivery vehicles (*Anupana*), and duration (*Sevana Maryada*), these formulations demonstrate targeted clinical efficacy.[2] However, the international integration of metal-containing therapeutics faces significant pharmacovigilance scrutiny due to recurring reports of heavy metal toxicity associated

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with substandard commercial products.[3] This underscores the necessity to distinguish between spurious preparations and classically standardized medicines through systematic toxicological and analytical profiling.

Crucially, total elemental metal content does not directly indicate biological toxicity; rather, bio-accessibility and physiological behavior are dictated by solid-state chemical speciation, crystal lattice morphology, and particulate dimensions. For instance, *Naga Bhasma* forms a bio-inert lead sulfide (PbS) matrix that remains clinically safe within therapeutic ceilings.[4] Similarly, *Rasa Sindoor* sublimates into an insoluble alpha-crystalline mercuric sulfide (HgS) lattice, effectively mitigating the toxicological risks characteristic of elemental or organic mercury.[5] Therefore, objective safety evaluations must assess the finalized, molecularly transformed pharmaceutical matrix rather than raw elemental inputs.

Swarna Vanga is an archetypal *Kupipaka Rasayana* (graduated sand-bath sublimated formulation) primarily prepared from purified *Vanga* (tin), *Parada* (mercury), and *Gandhaka* (sulfur), alongside auxiliary materials such as *Navasadara* (ammonium chloride) and *Suryakshara* (potassium nitrate).[6-10] It is traditionally indicated in *Prameha* (urinary and metabolic disorders), *Vatavyadhi* (neuromuscular disorders), *Kasa-Shwasa* (cough and dyspnea), *Dhatukshaya* (tissue depletion), *Svapnadosha* (nocturnal emissions), *Shukrameha* (spermatorrhea), and *Shvetapradara* (leukorrhea).[6,] Modern preclinical investigations have corroborated its mild hypoglycemic actions, alloxan-protective potential,[11] and baseline organ safety within therapeutic ranges (12.5–25 mg/100 g body weight),[12] alongside significant germinal epithelial regeneration.[13] Clinically, *Swarna Vanga* has shown versatile multi-systemic efficacy, functioning as a cytoprotective *Rasayana* adjuvant to alleviate chemotherapy-induced adverse effects, an endocrine modulator in primary hypothyroidism, and a key agent in *Vajikarana* regimens for reversing seminal disorders like azoospermia.[13]

Synthesized through sequential thermal processing that ensures structural stabilization of the final compound, operational protocols nevertheless vary across classical compendia.[3,6,10] In the present study, two distinct variations were synthesized: *Swarna Vanga* 1 (SV1), prepared according to Rasamrita,[14] and *Swarna Vanga* 2 (SV2), prepared according to Rasatantrasara evam Siddhaprayoga Samgraha.[15] Although both formulations share a similar metallurgical composition, variations in auxiliary reagent inclusion (such as *Suryakshara* in SV2) and trituration procedures may influence their physicochemical characteristics.[16] Despite their extensive therapeutic use, comparative experimental data evaluating whether these divergent classical manufacturing methodologies alter safety margins remain scarce. To establish objective safety benchmarks within modern biomedical and global regulatory frameworks, the present study comparatively evaluated the acute oral toxicity profiles of SV1 and SV2 in strict accordance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (OECD, 2001).[17]

MATERIALS AND METHODS

Study Design and Ethical Approval

The study was conducted according to a pre-approved experimental protocol and applicable institutional standard operating procedures (SOPs). The acute oral toxicity evaluation was performed in accordance with the OECD Test Guideline 423, Acute Oral Toxicity - Acute Toxic Class Method. OECD TG 423 is a sequential procedure based on predefined dose levels and the use of three animals of a single sex at each step, with subsequent dose selection determined by mortality and/or moribund status.[17]

The experimental study was conducted at the Department of Pharmacy, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara, Gujarat, India, in a laboratory animal facility registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),[18] Government of India, under Registration No. 404/PO/Re/S/01/CPCSEA, dated 28 October 2015. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of The Maharaja Sayajirao University of Baroda under approval No. MSU/IAEC/2025-26/2503, dated 15 November 2025. All experimental procedures were conducted in accordance with applicable institutional and national requirements for the care and use of laboratory animals. The study design incorporated the principles of replacement, reduction, and refinement wherever applicable.

Classical References and Preparation of Test Samples

Two distinct formulations of *Swarna Vanga* were synthesized independently in accordance with classical Ayurvedic compendia. *Swarna Vanga* 1 (SV1) was prepared following the protocol described in Rasamrita,[14] whereas *Swarna Vanga* 2 (SV2) was synthesized according to Rasatantrasaar evam Siddhaprayoga Samgraha.[15]

Before pharmaceutical synthesis, all raw starting materials underwent standardized classical *Shodhana* (purification/detoxification) protocols. *Vanga* (tin) was purified following classical procedures detailed in Rasatarangini,[19-20] *Parada* (mercury) and *Gandhaka* (sulfur) were purified according to Rasatarangini.[21,22]

For the synthesis of both formulations, prescribed quantities of *Shuddha Vanga* were transferred into an iron ladle (*Lauha Darvi*) and heated until complete liquefaction. *Shuddha Parada* was added to the molten tin, followed by continuous mechanical trituration for approximately 1 h to obtain a uniform amalgam (*Pishti*). The resulting *Pishti* was levigated with fresh lemon juice (*Nimbu Svarasa*) and rock salt (*Saindhava Lavana*) to eliminate surface impurities, washed thoroughly with distilled water, and dried completely.

The dried amalgam was transferred into an iron mortar (*Lauha Khalva Yantra*) and triturated with the prescribed stoichiometric quantity of *Shuddha Gandhaka* until achieving *Nischandratva* (complete loss of metallic luster), yielding a fine *Kajjali*. Subsequently, processing additives were incorporated: *Navasadara* (ammonium chloride) was added to the *Kajjali* for SV1, whereas both *Navasadara* and *Suryakshara* (potassium nitrate) were incorporated for SV2, followed by brief trituration to ensure homogeneous blending.

The respective homogenized intermediate mixtures were transferred into green glass beer bottle (*Kupi*), wrapped uniformly in seven sequential layers of clay-smeared cotton cloth (*Kapadamitti*), and positioned centrally within a sand-bath apparatus (*Valuka Yantra*). The assemblies were placed in a heating furnace (*Bhrashtri*) and subjected to a controlled, progressive thermal gradient (*Kupipakwa* processing) until the complete cessation of white and sulfur fumes, signaling the terminal phase transition. Following natural cooling of the apparatus to ambient room temperature, the *Kupi* were carefully broken, and the characteristic mosaic-gold/yellow crystalline products settled at the bottom of the bottle (*Talastha*) were harvested, weighed, and stored in clean, dry, airtight amber glass containers designated as SV1 and SV2 for subsequent evaluation.

The relative proportions of ingredients used for the preparation of SV1 and SV2 are presented in Table 1.

Figures 1 and 2 depict the prepared samples of SV1 and SV2.

Experimental Animals

Healthy female Sprague–Dawley rats aged 8–12 weeks and weighing 150–350 g were selected for the study. Animals were examined before inclusion in the experiment, and only healthy animals without observable clinical abnormalities were enrolled. A total of 27 female rats were included in the study. The animals were allocated to the vehicle control and test-treatment groups as described in Table 2. The use of female animals was consistent with the OECD TG 423 approach, which specifies that testing with one sex, usually females, is generally sufficient for the acute toxic class method.[17]



Figure 1: *Swarna Vanga* 1

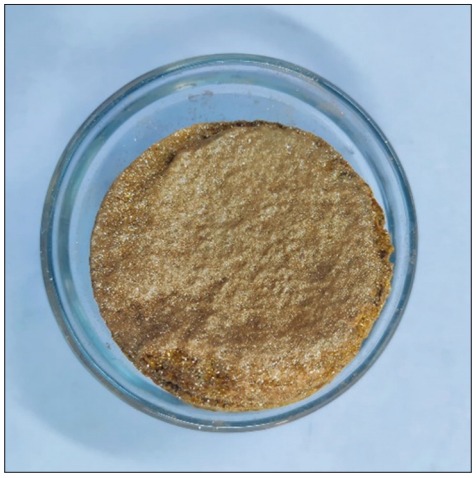


Figure 2: *Swarna Vanga* 2

Table 1: Composition of *Swarna Vanga* preparations according to the two classical references

S. No.	Ingredient	SV1	SV2
1	*Shuddha Parada* (Mercury)	1 Part	5 Parts
2	*Shuddha Vanga* (Tin)	2 Parts	5 Parts
3	*Shuddha Gandhaka* (sulfur)	1 Part	5 Parts
4	*Navasadara* (Ammonium Chloride)	1 Part	4 Parts
5	*Suryakshara* (Potassium Nitrate)	-	1 Part

SV1: *Swarna Vanga* 1, SV2: *Swarna Vanga* 2

Housing and Feeding Conditions

The animals were housed in appropriate polypropylene cages under controlled laboratory conditions. The animal room was maintained at a temperature of 22 ± 3°C with a controlled 12-h light/12-h dark cycle. Animals were provided a standard laboratory pellet diet and had free access to potable drinking water *ad libitum*, except during the specified pre-dose fasting period. Animal husbandry, handling, feeding,

Table 2: Experimental groups and dose levels

Group	Treatment	Dose level	Number of animals
I	Vehicle control	1% CMC	3
II	SV1	5 mg/kg, p.o.	3
	SV1	50 mg/kg, p.o.	3
	SV1	300 mg/kg, p.o.	3
	SV1	2000 mg/kg, p.o.	3
III	SV2	5 mg/kg, p.o.	3
	SV2	50 mg/kg, p.o.	3
	SV2	300 mg/kg, p.o.	3
	SV2	2000 mg/kg, p.o.	3

CMC: Carboxymethylcellulose, SV1: *Swarna Vanga* 1, SV2: *Swarna Vanga* 2

and experimental procedures were performed in accordance with applicable institutional requirements and the regulatory framework governing the care and use of laboratory animals in India. The CCSEA framework emphasizes appropriate animal care and the principles of replacement, reduction, and refinement in animal experimentation.[18]

Acclimatization and Animal Identification

All animals were acclimatized to the experimental environment for a minimum of 7 days before administration of the test substances. During acclimatization, animals were observed daily for general health and clinical abnormalities. Animals were individually identified by appropriate tail marking using permanent markers. Animals assigned to SV1 groups were identified using a blue marker, those assigned to SV2 groups using a green marker, and vehicle-control animals using a red marker. Each cage was additionally labeled with the corresponding treatment and dose level to ensure accurate identification throughout the study. Before dosing, animals were examined to confirm their suitability for inclusion in the study. Body weight was recorded for dose calculation.

Preparation of Test Suspensions

The respective *Swarna Vanga* preparations were finely powdered using a clean mortar and pestle and suspended in 1% carboxymethylcellulose (CMC) immediately before administration. For administration, the required quantity of each test preparation was dispersed in the vehicle to obtain the intended dose concentration. For the 5 and 50 mg dose preparations, the required quantity was suspended in 5 mL of 1% CMC, whereas for the 300 and 2000 mg dose preparations, the required quantity was suspended in 20 mL of 1% CMC. The suspensions were freshly prepared and adequately mixed before administration to minimize sedimentation and ensure uniformity of the administered preparation. The vehicle-control animals received an equivalent administration of 1% CMC without the test substance.[23]

Dose Calculation and Administration

The dose administered to each animal was calculated according to its individual body weight and expressed as mg/kg body weight. The appropriate quantity of the test suspension was administered orally to the respective animal. Before dosing, animals were fasted overnight, with water available *ad libitum*. Following administration, food was returned approximately 3 - 4 h after dosing. The test preparations were administered by the oral route (per os, p.o.) using an appropriate oral feeding needle. The administration was performed carefully to minimize the risk of injury or aspiration.

Acute Oral Toxicity Procedure

Acute oral toxicity testing was performed using the sequential procedure described in OECD TG 423. Three animals of the selected sex were used at each dose level. The initial dose and subsequent dose levels were selected according to the predefined OECD dose sequence and the observed mortality and/or moribund status at each step.[17] For each preparation, SV1 and SV2 were evaluated independently using the dose sequence specified in the approved study protocol. The dose levels investigated were 5, 50, 300, and 2000 mg/kg body weight.

Animals were observed individually following administration and periodically during the initial post-dose period and subsequently throughout the observation period. Particular attention was given to mortality and treatment-related clinical signs, including changes in general behavior, activity, posture, gait, response to external stimuli, respiratory pattern, autonomic activity, neurological behavior, gastrointestinal signs, and other observable abnormalities. The onset, severity, duration, progression, and reversibility of any clinical signs were recorded. Animals were also monitored for evidence of morbidity or severe distress. Any animal meeting predefined humane-endpoint criteria was to be euthanized in accordance with the approved institutional procedure and considered in the interpretation of the study according to OECD TG 423.[17]

Observation Period and Clinical Examination

Animals were observed closely following administration and subsequently throughout the predefined observation period. Clinical observations were recorded systematically for each animal.

The observation parameters included:

- Mortality and moribund status
- General appearance and behavior

- Locomotor activity
- Posture and gait
- Response to external stimuli
- Respiratory pattern
- Salivation and lacrimation
- Tremors, convulsions, or other neurological manifestations
- Changes in feeding or drinking behavior
- Fecal and urinary abnormalities
- Any other treatment-related clinical signs.

The presence, absence, onset, duration, severity, and recovery of clinical signs were documented for individual animals.

Body-weight Assessment

Individual body weights were recorded before administration of the test substance and subsequently during the observation period at the predefined study intervals. Changes in body weight were monitored as an additional indicator of the general health status and potential treatment-related effects.

Outcome Assessment and Toxicity Classification

The acute oral toxicity profile of SV1 and SV2 was evaluated on the basis of mortality, moribund status, clinical observations, changes in body weight and, where applicable. The resulting toxicity category was interpreted according to the decision criteria and classification framework specified in OECD TG 423. The findings obtained for SV1 and SV2 were compared to determine whether differences in their classical pharmaceutical preparation were associated with differences in the observed acute oral toxicity profile.

OBSERVATIONS AND RESULTS

Study Progression and Survival

The acute oral toxicity evaluation of *Swarna Vanga* preparations was conducted according to the sequential dosing approach specified in OECD Test Guideline 423. The initial administration of SV1 and SV2 was performed at dose levels of 5 and 50 mg/kg body weight on 15 June 2026. Based on the observations from the preceding dose levels, subsequent dose levels of 300 and 2000 mg/kg body weight were administered on 17th June 2026.

All animals were monitored throughout the study period for mortality, treatment-related clinical signs, changes in general behavior, locomotor activity, neurological manifestations, respiratory abnormalities, gastrointestinal changes, and other observable signs of toxicity. No mortality was recorded in any of the SV1, SV2, or vehicle-control animals during the observation period. No animal required premature termination because of treatment-related severe toxicity or moribundity.

Changes in Body Weight

Individual body weights were recorded at baseline and at the end of the observation period. The mean body-weight values, standard error of the mean (SEM), mean change from baseline, paired t-test statistic, and corresponding *P* values for animals treated with SV1 and SV2 are presented in Table 3.

Animals administered SV1 at 5 mg/kg showed an increase in mean body weight from 221.33 ± 5.55 g on day 1 to 250.00 ± 6.81 g on day 14, corresponding to a mean increase of 28.67 ± 8.11 g ($P = 0.072$). At 50 mg/kg, mean body weight increased from 257.00 ± 16.92 g to 267.00 ± 17.16 g, with a mean increase of 10.00 ± 1.53 g ($P = 0.023$). At 300 mg/kg, mean body weight increased from 191.00 ± 8.51 g to 214.33 ± 9.56 g, corresponding to a mean increase of 23.33 ± 2.60 g ($P = 0.012$). At 2000 mg/kg, mean body weight showed a slight decrease from 256.33 ± 15.41 g to 250.33 ± 4.70 g, representing a mean change of −6.00 ± 11.59 g ($P = 0.656$).

For SV2, animals administered 5 mg/kg showed an increase in mean body weight from 195.67 ± 11.62 g to 213.00 ± 10.07 g, with a mean increase of 17.33 ± 5.81 g ($P = 0.096$). At 50 mg/kg, mean body weight increased from 209.00 ± 5.51 g to 212.67 ± 3.38 g, corresponding to a mean increase of 3.67 ± 4.06 g ($P = 0.461$). At 300 mg/kg, mean body weight increased from 196.67 ± 11.56 g to 201.33 ± 15.02 g, with a mean increase of 4.67 ± 9.70 g ($P = 0.678$). At 2000 mg/kg, mean body weight decreased from 210.00 ± 7.37 g to 171.33 ± 23.13 g, corresponding to a mean change of −38.67 ± 26.03 g ($P = 0.276$).

In the vehicle-control group receiving 1% CMC (1 mL/kg), mean body weight decreased from 176.33 ± 0.56 g to 169.00 ± 14.53 g, corresponding to a mean change of −7.33 ± 10.73 g ($P = 0.565$). Statistically significant within-group differences in body weight were observed in the SV1 groups administered 50 mg/kg ($P = 0.023$) and 300 mg/kg ($P = 0.012$). No statistically significant difference between baseline and terminal body weight was observed in the remaining SV1, SV2, or vehicle-control groups. The complete statistical analysis is presented in Table 3.

Clinical Observations

Clinical observations were performed at predefined intervals following oral administration and throughout the observation period. Animals were evaluated for changes in skin and fur, eyes, mucous membranes, salivation, respiratory pattern, locomotor activity, grooming behavior, neurological manifestations, gastrointestinal signs, food and water intake, lethargy, sleep, coma, mortality, and any other abnormal clinical findings. No treatment-related clinical signs were observed in animals administered either SV1 or SV2 at any of the investigated dose levels (5, 50, 300, and 2000 mg/kg body weight). The animals

Table 3: Effect of SV1 and SV2 administration on body weight of rats

Treatment group	Dose (mg/kg)	Day 1, Mean±SEM (g)	Day 14, Mean±SEM (g)	Mean difference±SEM (g)	t value	*P*-value	Significance
SV1	5	221.33±5.55	250.00±6.81	28.67±8.11	−3.535	0.072	NS
	50	257.00±16.92	267.00±17.16	10.00±1.53	−6.547	0.023	Significant
	300	191.00±8.51	214.33±9.56	23.33±2.60	−8.963	0.012	Significant
	2000	256.33±15.41	250.33±4.70	−6.00±11.59	0.518	0.656	NS
SV2	5	195.67±11.62	213.00±10.07	17.33±5.81	−2.982	0.096	NS
	50	209.00±5.51	212.67±3.38	3.67±4.06	−0.904	0.461	NS
	300	196.67±11.56	201.33±15.02	4.67±9.70	−0.481	0.678	NS
	2000	210.00±7.37	171.33±23.13	−38.67±26.03	1.486	0.276	NS
Vehicle control	1% CMC, 1 mL/kg	176.33±0.56	169.00±14.53	−7.33±10.73	0.684	0.565	NS

Values are expressed as mean±SEM; n=3 animals per dose group. Within-group comparisons between day 1 and day 14 were performed using a paired t-test. NS, not statistically significant. CMC: Carboxymethylcellulose, SEM: Standard error of the mean, SV1: *Swarna Vanga* 1, SV2: *Swarna Vanga* 2

Table 4: Clinical observations following oral administration of SV1 and SV2

Observation parameter	0.5 h	1 h	2 h	4 h	12 h	24 h	Day 2	Days 3–14
Skin and fur changes	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N
Mucous membranes	N	N	N	N	N	N	N	N
Tremors	N	N	N	N	N	N	N	N
Convulsions	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N
Abnormal sleep/somnolence	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N
Locomotor activity	N	N	N	N	N	N	N	N
Grooming	N	N	N	N	N	N	N	N
Food consumption	N	N	N	N	N	N	N	N
Water intake	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N
Any other abnormal finding	N	N	N	N	N	N	N	N

N: No abnormal observation recorded

remained clinically normal throughout the observation period. No abnormalities were observed in skin and fur, eyes, mucous membranes, respiration, locomotor activity, grooming, food consumption, or water intake. No episodes of tremors, convulsions, abnormal salivation, diarrhea, marked lethargy, abnormal sleep, coma, or other overt neurological or behavioral abnormalities were observed. Similarly, no treatment-related mortality occurred in any of the experimental or vehicle-control groups. The detailed clinical observations recorded at 0.5, 1, 2, 4, 12, and 24 h after administration and subsequently during the observation period are summarized in Table 4.

DISCUSSION

The present investigation comparatively evaluated the acute oral toxicity of two classical *Swarna Vanga* - SV1 (prepared according to Rasamrita) and SV2 (prepared according to Rasatantrasaar evam Siddhaprayoga Samgraha) in accordance with OECD Test Guideline 423 (Acute Toxic Class Method). The principal finding was the complete absence of treatment-related mortality, moribundity, or overt signs of systemic clinical toxicity following single-dose oral administration of either formulation up to the limit dose of 2000 mg/kg body weight (OECD, 2001). Comprehensive clinical examinations

revealed no treatment-induced alterations in autonomic, behavioral, neuromuscular, or physiological parameters across all dose cohorts (5, 50, 300, and 2000 mg/kg). Parameters including locomotion, grooming, respiratory rate/pattern, pupillary reflexes, salivation, lacrimation, and gastrointestinal output remained unperturbed throughout the 14-day observation window. Consequently, both finished preparations can be classified under the Globally Harmonized System (GHS) Category 5/Unclassified (LD 50 > 2000 mg/kg), indicating a high threshold of acute oral tolerability.[24]

Acute toxicity profile and regulatory interpretation

The absence of mortality and moribundity at the highest tested dose (2000 mg/kg) positions both SV1 and SV2 within the Globally Harmonized System (GHS) Category 5 or unclassified range for acute oral toxicity, as per OECD TG 423 decision logic. Under this guideline, substances that do not produce mortality at 2000 mg/kg are generally considered to have low acute oral hazard, although this does not preclude the need for further toxicological characterization for repeated-dose or chronic exposure scenarios. The concordance between mortality data and the absence of treatment-related clinical signs – including neurological, respiratory, gastrointestinal, and behavioral abnormalities – strengthens the interpretation that neither preparation elicited acute systemic toxicity under the experimental conditions employed.

The experimental and analytical findings of the present study align closely with earlier preclinical investigations into the safety and pharmacological efficacy of *Swarna Vanga*. Previous toxicity profiles documented by Sharma *et al.*[12] and Singh *et al.*[13] demonstrated that therapeutic doses (12.5–100 mg/100 g body weight in rats) elicited no histopathological abnormalities across major vital organs, confirming that the classical *Kupipakwa* sublimation process effectively renders the constituent heavy metals chemically stable and biocompatible. Mild degenerative changes in hepatic and intestinal tissues emerged only under excessive, supratherapeutic doses, underscoring the established safety margin of the formulation within rational clinical limits. Furthermore, prior bio-evaluations highlighting significant normoglycemic blood sugar regulation, alloxan-protective trends, and testicular germinal epithelial regeneration corroborate the multi-systemic therapeutic efficacy of this herbo-metallic compound across metabolic and reproductive pathologies. However, the present study extends these findings by directly comparing two textually distinct formulations under a standardized regulatory framework, thereby contributing to the evidence base for classical pharmaceutical variability and acute tolerability.

Comparative Physicochemical Speciation and Toxicological Correlation

Despite text-specific differences in precursor stoichiometry and the inclusion of *Suryakshara* (KNO_3) alongside *Navasadara* (NH_4Cl) in SV2 (Rasatantrasaar evam Siddhaprayoga Samgraha) versus SV1 (Rasamrita), both formulations exhibited identical acute oral safety profiles (GHS Category 5/Unclassified, LD50 > 2000 mg/kg). This toxicological equivalence is directly explained by their convergent solid-state speciation and micromorphology. X-ray diffraction (XRD) and scanning electron microscopy (SEM) confirmed that both preparations undergo complete phase transformation into a well-defined crystalline matrix dominated by tin disulfide (SnS_2), which aligns with previously reported characteristic XRD diffraction patterns validating the uniform crystallization of stannic sulfide lattices across diverse classical manufacturing protocols.[9] Inductively coupled plasma mass spectrometry (ICP-MS) corroborated that tin and sulfur constitute the bulk stoichiometric framework in both SV1 (Sn: 55.32%, S: 38.69%) and SV2 (Sn: 40.96%, S: 44.59%),[25] consistent with established stoichiometric ranges where tin and sulfur represent the predominant elemental matrix of finished *Swarna Vanga*.[9] The elevated potassium (8.26%), sodium (6.53%), and iron (3.08%) content in SV2 reflects the thermal mineral retention from *Suryakshara* and classical processing aids, yet these minor phases did not induce differential acute reactivity.[25]

Critically, elemental mercury (*Parada*) functioned strictly as a transient reaction flux and mineralizing vehicle rather than a structural constituent of the final product. Initial trituration (*Kajjali*) enabled mercury to disrupt the metallic tin lattice and drive nanoscale intimacy with sulfur, mechanically dividing the tin into micro and sub-micron fragments that maximize reactive surface area for subsequent sulfur binding. Experimental evidence demonstrates that incorporating mercury in at least a 1:1 or 1:1/2 ratio relative to tin is essential during the amalgam (*Dhatu Pishti*) phase to ensure complete reaction kinetics, maximum product yield, and standard golden crystalline luster without leaving unreacted tin metal. During the high-temperature *Kupipakwa* calcination gradient (>350–580°C), mercury catalyzed the solid-state crystallization of SnS_2 before largely volatilizing and sublimating out of the core reaction zone due to its high vapor pressure.[25] Consequently, residual mercury was reduced to trace levels (SV1: 909.92 ppm; SV2: 883 ppm),[25] representing a near-complete matrix clearance relative to starting inputs and mirroring baseline toxicological and analytical findings where mercury is either completely absent or present in negligible, non-toxic trace fractions.

From a toxicokinetic standpoint, the safety profile is governed by the solid-state speciation rather than raw elemental inputs. Crystalline metal chalcogenides (SnS_2) possess an exceptionally low solubility product and chemical stability in physiological media. Particle size analysis (PSA) revealed submicron distributions for both SV1 (0.18–0.56 µm) and SV2 (0.18–0.60 µm),[25] which strongly corroborates previous laser diffraction particle size evaluations of *Swarna Vanga* showing an average

particulate diameter of 541.23 nm with over 97% of particles distributed between 536.0 nm and 729.2 nm.[8,25] While submicron domains enhance surface contact and facilitate bio-assimilation, the chemical inertness of the insoluble SnS_2 lattice impedes ionic dissociation in gastrointestinal fluids, markedly suppressing systemic bioaccessibility and membrane permeability. Preclinical and sub-acute toxicity evaluations corroborate this non-toxic visceral profile at therapeutic doses (12.5–25 mg/100 g body weight/day), showing that classically synthesized *Swarna Vanga* does not induce histopathological abnormalities across major organs.[12,13] Concurrently, this stable particulate speciation underpins its validated therapeutic activities – such as mild hypoglycemic control, germinal epithelial regeneration, cytoprotective *Rasayana* efficacy against chemotherapy adverse effects, and endocrine modulation in metabolic syndromes.[13] These analytical findings demonstrate that classical *Kupipakwa* thermal processing buffers precursor variations toward a uniform, toxicologically inert SnS_2 phase. While these data establish the acute safety of both variants, high-resolution speciation and repeated-dose sub-chronic toxicity studies remain essential to evaluate cumulative pharmacokinetics and long-term biological safety.

Body-weight Changes: Biological and Statistical Considerations

Body-weight trajectories in the present study were variable and did not demonstrate a consistent dose-dependent adverse pattern. Animals receiving SV1 at 5, 50, and 300 mg/kg exhibited mean body-weight increases, with a small decrease observed only at 2000 mg/kg. A similar pattern was noted for SV2, with increases at lower doses and a reduction at 2000 mg/kg. Statistically significant within-group increases were observed in SV1 at 50 and 300 mg/kg; however, these represent weight gains rather than losses and, in isolation, do not indicate toxicity.

The SV2 2000 mg/kg group showed a mean body-weight reduction of 38.67 ± 26.03 g, which was not statistically significant ($P = 0.276$) and was accompanied by substantial inter-animal variability (large SEM). Importantly, the vehicle-control group also exhibited a small reduction in mean body weight, indicating that such fluctuations can occur independently of test-article administration. Taken together, these findings do not support a treatment-related adverse effect on body weight attributable to either SV1 or SV2.

Interpretation of statistical significance in this context requires caution due to the small sample size inherent to OECD TG 423, which employs three animals per sex per step. As emphasized by the ARRIVE 2.0 guidelines,[26] P values derived from very small groups should not be interpreted in isolation from effect magnitude, direction, biological plausibility, and consistency with other endpoints. Transparent reporting of exact sample sizes, descriptive statistics, and limitations is essential to avoid over-interpretation of marginally significant findings in small-group toxicology studies.

Study Limitations and Future Directions

Several limitations should be considered when interpreting the present findings. First, the sequential design of OECD TG 423 results in small group sizes (n = 3/sex/step), which limits statistical power for continuous endpoints such as body weight and reduces sensitivity to subtle treatment-related effects. Second, the study focused exclusively on acute oral toxicity and did not evaluate repeated-dose toxicity, cumulative exposure, reproductive toxicity, genotoxicity, or organ-specific endpoints. Consequently, the findings cannot be extrapolated to chronic administration or prolonged therapeutic use. Third, while the absence of mortality and overt clinical signs is reassuring, the dataset does not establish the absence of subclinical organ toxicity. Incorporation of hematological, biochemical, and histopathological endpoints in future repeated-dose studies would provide a more comprehensive assessment of systemic safety. Finally, detailed comparative pharmaceutico-analytical characterization of SV1 and SV2 is essential to determine whether differences in classical composition and processing translate into meaningful differences in physicochemical properties, bioavailability, or long-term biological effects.

CONCLUSION

This study demonstrates that both classical *Swarna Vanga* formulations – SV1 (Rasamrita) and SV2 (Rasatantrasaar evam Siddhaprayoga Samgraha) – demonstrated a high threshold of acute oral tolerability meeting GHS Category 5/Unclassified criteria (LD 50 > 2000 mg/kg) under OECD TG 423, producing zero mortality, clinical abnormalities, or adverse body-weight alterations. Analytical characterization (XRD, SEM, PSA, and ICP-MS) confirmed that high-temperature *Kupipakwa* calcination effectively buffers textual precursor differences, driving both formulations toward a shared, chemically inert, submicron crystalline tin disulfide (SnS_2) lattice. Mercury (*Parada*) functioned strictly as a transient mineralizing flux – catalyzing SnS_2 crystallization before thermally sublimating out of the reaction zone to leave only trace residuals – while the high thermodynamic stability and low solubility product of the resulting SnS_2 matrix minimized gastrointestinal dissolution and systemic bioaccessibility. These findings validate the acute safety and chemical speciation-driven detoxification of classical *Swarna Vanga*, establishing an empirical baseline that warrants subsequent repeated-dose sub-chronic toxicity, histopathological, and pharmacokinetic studies to evaluate long-term systemic safety

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