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Biphasic Neurobehavioural Response and Preliminary Safety Profile of Swarna Bhasma (Traditional Nanogold) in *Drosophila melanogaster*

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Abstract

Swarna Bhasma (SB) is a traditionally processed gold-based Ayurvedic nanomedicine used for rejuvenative and neurocognitive purposes. This study evaluated the developmental, neurobehavioural, metabolic, oxidative, morphological, and intestinal effects of SB administered with its classical adjuvants, honey and ghee, in *Drosophila melanogaster*. First-instar Canton-S larvae were exposed through diet to SB at 0.6, 1.25, 2.5, 5.0, or 10.0 µg/mL, alongside plain and vehicle controls. Feeding, larval growth, pupation, adult emergence, sex ratio, locomotor performance, glucose, TAG, total protein, reactive oxygen species, nitric oxide, adult morphology, and intestinal integrity were assessed. SB did not alter food intake, developmental timing, total protein content, gross adult morphology, or intestinal epithelial integrity. A biphasic dose-response relationship was observed. The 2.5 µg/mL produced the most favourable profile, increasing larval weight and adult emergence and improvement in adult negative geotaxis. Lower concentrations enhanced larval crawling, whereas 5-10 µg/mL impaired larval and adult locomotor performance. At higher concentrations, adult emergence declined, glucose increased, and TAG decreased at 10 µg/mL. ROS increased at 5 µg/mL, while NO decreased at 0.6 and 10 µg/mL which indicate a biphasic response, with favourable neurobehavioural and developmental effects at an intermediate concentration but functional, metabolic, and redox alterations at higher exposures.

Major Findings: 1) 2.5 µg/mL showed the most favourable profile, increasing larval weight by 13%, adult emergence by 22%. 2) 25% reduction in TAG at 10 µg/mL, and 37% increase in ROS at 5 µg/mL. 3) 5–10 µg/mL impaired larval and adult locomotion and reduced adult emergence. Gross adult morphology, and intestinal epithelial integrity remained unaffected.

Keywords: Ayurveda, Dose-response Relationship, *Drosophila melanogaster*, Gold-based Nanomedicine, Hormesis, Metabolic Homeostasis, Neurobehavioural Toxicity, *Swarna Bhasma*

1. Introduction

Swarna Bhasma (SB) is a systematically processed gold-based formulation traditionally used in rejuvenative and pediatric practices, with purported benefits for cognition, physical strength, immune function, growth and development[1]. Contemporary physicochemical characterisation studies have been carried out earlier. SB has been reported to contain gold in crystalline and nanostructured forms produced through repeated pharmaceutical processing like purification, trituration, and controlled incineration with evidence of physicochemical stability and a distinct material profile compared to conventionally synthesised gold nanoparticles[2].

Preclinical investigations have reported encouraging biocompatibility findings for SB, including hemocompatibility, an absence of overt toxicity within the tested therapeutic dose range, and potential behavioural or neuroprotective effects in zebrafish and rodent models[3-5]. A recent study further demonstrated the neuroprotective activity of SB in a rotenone-induced

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Parkinson's-like model, increasing interest in its potential neuromodulatory properties[6]. Nevertheless, the available studies have predominantly evaluated therapeutic efficacy under disease conditions or assessed limited toxicological endpoints. Consequently, the concentration-dependent safety limits and whole-organism toxicological profile of SB under normal physiological conditions remain poorly defined. Therefore, a systematic organism-level dose-response assessment encompassing survival, development, metabolic homeostasis, redox balance, tissue integrity, and neurobehavioural function is needed to define the biological safety window of SB and distinguish potentially beneficial effects from toxic responses.

Drosophila melanogaster provides a robust whole-organism platform for addressing this knowledge gap because of its rapid development, well-characterised genetics, conserved metabolic as well as stress-response pathways, and established assays for developmental toxicity, neuromuscular functions, and neurobehavioural performance[7,8]. Larval crawling and adult negative geotaxis are sensitive indicators of locomotor function and sensorimotor coordination, whereas dye-based feeding assays, biochemical analyses, and tissue-staining techniques facilitate the evaluation of food intake, energy metabolism, redox status, and intestinal epithelial integrity[9-11]. Previous studies in *Drosophila* model have demonstrated that metallic and mineral nanoparticles can produce concentration-dependent alterations in survival, development, morphology, locomotion, carbohydrate homeostasis and oxidative balance[12-14], supporting the use of this model for distinguishing potentially beneficial responses from adverse effects of gold-based material.

Accordingly, the present study evaluated SB across a range of concentrations using integrated developmental, metabolic, oxidative, neurobehavioural, morphological and intestinal integrity endpoints. Particular attention was given to carbohydrate metabolism, lipid homeostasis, and oxidative-stress regulation because disturbances in these processes may serve as early and sensitive indicators of nanomaterial-induced toxicity[15]. To our knowledge, this is the first comprehensive investigation of dose-dependent safety profile and potential biphasic neurobehavioural effects of SB administered with its classical Ayurvedic adjuvants- honey and ghee (clarified butter) in *Drosophila melanogaster*. By integrating multiple functional and toxicological endpoints within a single *in vivo* model, this study seeks to define a biologically relevant safety window and establish an experimental framework for the mechanistic evaluation and translational development of SB as a traditional gold-based nanomedicine.

2. Materials and Methods

2.1 Preparation and Characterisation of *Swarna Bhasma* (SB)

Swarna Bhasma (batch no. P220300043), containing gold particles with a reported size range of 21-40 nm, was procured from a GMP-certified Ayurvedic manufacturer in Mumbai, India. The formulation was manufactured according to classical Ayurvedic procedures under established quality-control conditions[16]. Its physicochemical characteristics were evaluated using X-Ray Diffraction (XRD) and Fourier-Transform Infrared (FTIR) spectroscopy. XRD analysis was performed using a Rigaku Ultima IV diffractometer with Cu Kα radiation (λ = 0.154 nm). Diffraction patterns were recorded over a 2θ range of 10°-80° at a scanning rate of 2°/min. For FTIR analysis, 3-4 mg of SB was mixed with approximately 30 mg of potassium bromide, compressed into a pellet, and analysed using a PerkinElmer FTIR spectrometer over the range of 4000-400 cm^{-1}. Before analysis, the KBr pellets were dried in a vacuum desiccator and briefly heated under an infrared lamp to minimise interference from adsorbed moisture. XRD was used to confirm the crystalline elemental-gold phase, whereas FTIR was used to evaluate detectable surface-associated functional groups or residual organic constituents.

2.1.1 Chemicals and Reagents

All chemicals and reagents were of analytical grade and used without further purification. Components of the standard *Drosophila* diet, including yeast, sucrose, cornmeal, agar, methylparaben as an antifungal preservative, and propionic acid as an antibacterial preservative, were procured from HiMedia Laboratories, India. Multifloral honey and ghee were of food-grade quality. Trypan blue, nitroblue tetrazolium (NBT), glacial acetic acid, glycerol, and the remaining laboratory reagents were also obtained from HiMedia Laboratories, India.

2.2 *Drosophila* Strain and Maintenance

Wild-type Drosophila melanogaster (Canton-S strain) obtained from the Drosophila Stock Center, Mysore, India,

was used in this study. Stock cultures were maintained at 22 ± 1°C and approximately 70% relative humidity under a 12 h light/12 h dark photoperiod. Flies were reared on a standard cornmeal-yeast-agar medium containing yeast, sucrose, cornmeal, and agar, supplemented with methylparaben (nipagin) and propionic acid as antimicrobial preservatives. Under these conditions, development from egg to adult required approximately 10 days. All experiments were conducted using healthy flies reared under the same standardised conditions.

2.3 Dose Preparation and Exposure Protocol

Five experimental diets containing SB were prepared at concentrations of 0.6, 1.25, 2.5, 5.0, and 10.0 µg SB per mL of food. These concentrations were selected based on Ayurvedic dosage recommendations[17], and preliminary optimisation experiments with 2.5 µg/mL approximating a middle dose for flies, and to investigate biphasic effects. SB powder was suspended in honey-ghee mixture (honey:ghee ratio 1:0.25) with 0.1% DMSO as dispersant[18]. The suspension was sonicated on ice for 30 min (20 s pulses at 30% amplitude) for uniform distribution. This mixture was blended into cooled molten fly food before solidification to create the SB-infused diet.

Two control diets were prepared with five SB diets: A plain control without additions, and a vehicle control with honey-ghee-DMSO mixture excluding SB. For each group, comprising five SB concentrations, vehicle control, and plain control, first-instar larvae (24-30 h old, n≈100 per vial) were transferred to food and reared until adult emergence under described conditions. Larvae were exposed to SB (or control diets) from early development through metamorphosis. SB ingestion per larva was not quantified; exposure was defined by SB concentration in diet. All experiments had three independent replicates per group.

2.4 Gustatory Behaviour

To evaluate the effect of SB on larval food intake, a dye-based feeding assay was performed according to Croset *et al.*[19], with minor modifications. Third-instar larvae were allowed to feed on food containing Brilliant Blue FCF for a defined period. After feeding, the larvae were thoroughly rinsed, homogenised, and the optical density of the homogenates was measured at 630 nm as an index of food consumption. The assay was performed in triplicate using independent larval cohorts.

2.5 Developmental and Morphological Assessments

Several developmental indices were monitored from larval to adult stages.

2.5.1 Larval Size and Weight

Wandering third-instar larvae were collected from each experimental group. Body length and width were measured using a calibrated microscope, and larval wet weight was determined in batches of 10 larvae.

2.5.2 Developmental Timing

The time to reach third instar was recorded (it was approximately 72 h for all groups).

2.5.3 Pupation and Adult Emergence

The numbers of pupae formed and adults emerged were recorded daily for each vial. Pupation and adult-emergence percentages were calculated relative to the initial number of larvae introduced into each vial: pupation (%) = number of pupae/initial number of larvae × 100, and adult emergence (%) = number of emerged adults/initial number of larvae × 100. For graphical comparison, these values were subsequently normalised to the untreated control, which was assigned a value of 100%[20]. Developmental delays and failures, including failure to pupate or emerge as an adult, were also recorded.

2.5.4 Adult Phenotype

Ten newly emerged adult flies were randomly selected from each experimental group and examined under a stereomicroscope for gross morphological abnormalities. The assessment included wing morphology; eye structure and pigmentation; and thoracic bristle patterns[21]. Morphological findings in SB-treated flies were compared with those in the untreated and vehicle-control groups to identify treatment-associated developmental abnormalities.

2.6 Preparation of Larval Homogenates

For each experimental group, 50 third-instar larvae were rinsed with distilled water and transferred to ice-cold homogenisation buffer consisting of 1× phosphate-buffered saline (PBS; pH 7.4), 1 mM phenylmethylsulfonyl fluoride, and 0.5 mM EDTA. The larvae were homogenised on ice at a tissue-to-buffer

ratio of 1:10 (w/v) using a glass homogeniser. The resulting homogenate was centrifuged at 10,000 × g for 10 min at 4°C, and the clarified supernatant was collected for immediate biochemical analysis. Total protein concentration was determined using the Bradford assay, and biochemical measurements were expressed relative to milligrams of total protein to account for differences in sample concentration.

2.7 Metabolic Assessments (Protein, Glucose and TAG)

Total protein, glucose, and TAG concentrations were quantified in clarified larval homogenates to assess the effects of SB on energy metabolism. Total protein was measured using the Bradford method, with bovine serum albumin as the calibration standard[22]. Glucose and TAG concentrations were determined colorimetrically using commercial Liquick Cor-GLUCOSE and Liquick Cor-TG assay kits, respectively (PZ Cormay, Poland), according to the manufacturer's instructions. Absorbance was measured using a microplate reader at the wavelengths specified for each assay. Glucose and TAG values were normalised to the total protein content of the corresponding samples and expressed per milligram of protein.

2.8 Oxidative Stress Assessments (Reactive Oxygen Species and Nitric Oxide)

Oxidative status was assessed by measuring Reactive Oxygen Species (ROS) and nitric oxide (NO).

2.8.1 ROS Measurement (NBT Reduction)

Superoxide-associated oxidative activity in larval haemolymph was assessed using the Nitroblue Tetrazolium (NBT) reduction assay, adapted from Sabat *et al.*[23]. Fifteen third-instar larvae from each experimental group were rinsed with Phosphate-Buffered Saline (PBS) to remove adhering food particles. The larval cuticle was punctured in the thoracic region using a sterile needle, and haemolymph was collected on ice to minimise melanisation. Five microlitres of pooled haemolymph was mixed with 10 µL of 1× PBS, followed by the addition of an equal volume (15 µL) of NBT working solution. The mixture was incubated at room temperature for 1 h in the dark. The reaction was terminated by adding 30 µL of glacial acetic acid, followed by centrifugation at maximum speed for 1 min. After the addition of 50% acetic acid, absorbance was measured at 595 nm using a microplate reader[24]. Higher absorbance was interpreted as greater NBT-reducing activity and used as a relative indicator of superoxide-associated oxidative status.

2.8.2 Nitric Oxide (NO)

Nitric oxide production was estimated indirectly by quantifying nitrite (NO_2^-), a stable oxidation product of nitric oxide, using the Griess reaction[25]. Clarified larval homogenate supernatant was treated with Griess reagent and incubated at room temperature. Absorbance was subsequently measured at 540 nm using a multimode microplate reader. Nitrite concentrations were calculated by interpolation from a nitrite standard curve ranging from 0 to 100 µM and expressed relative to the total protein content of the corresponding samples.

2.9 Behavioural Observations

Locomotor behaviour was assessed at two stages to determine neurobehavioural effects of SB.

2.9.1 Larval Crawling Assay

Third-instar larvae were individually placed at the centre of a Petri dish containing 2% agarose and marked with a calibrated grid. Each larva was allowed to crawl freely for 1 min while its movement was video-recorded. The crawling trajectory was traced from the recording, and the total distance travelled was determined using the calibrated grid. Crawling speed was calculated by dividing the distance travelled by the recording duration and was expressed in mm/s. Movement patterns were also examined for abnormalities, including excessive turning, reduced mobility, and hyperactivity[26].

2.9.2 Adult Climbing Assay (Negative Geotaxis)

Adult locomotor performance was assessed using a negative geotaxis assay adapted from Manjila and Hasan[27]. Groups of 10 adult flies aged 3-5 days were transferred to a transparent glass cylinder measuring 25 cm in height and allowed to acclimatise for 10 min. The flies were gently tapped to the bottom of the cylinder, and the number that climbed above a 6 cm threshold within 10 s was recorded. Climbing performance was calculated as the percentage of flies crossing the threshold: climbing performance (%) = number of flies crossing 6 cm/total number of flies ×

100. Three trials were conducted for each experimental group, and the mean percentage was used as an indicator of locomotor function and neuromuscular coordination.

2.10 Intestinal Tissue Integrity (Trypan Blue Exclusion)

Overt tissue damage in third-instar larvae following SB exposure was evaluated using a Trypan Blue exclusion assay adapted from Krebs and Feder[28]. Five larvae from each experimental group were rinsed with 1 × Phosphate-Buffered Saline (PBS) to remove adhering food particles and incubated in 0.02% Trypan Blue solution for 30 min in the dark. The larvae were subsequently washed thoroughly with PBS to remove excess dye and examined under a stereomicroscope. Images were captured to document the presence and distribution of blue staining in the intestinal region. Blue-stained areas were interpreted as membrane-compromised or damaged tissue, whereas the absence of staining indicated no detectable overt tissue damage under the assay conditions.

2.11 Sex Ratio Determination

The sex of newly emerged adult flies was recorded to evaluate potential sex-specific effects of SB exposure. Males and females were distinguished based on abdominal pigmentation and the presence of sex combs on the forelegs. The numbers of males and females in each experimental group were recorded, and the sex ratio was expressed as the male-to-female ratio. Deviations from the expected 1:1 ratio were evaluated as potential

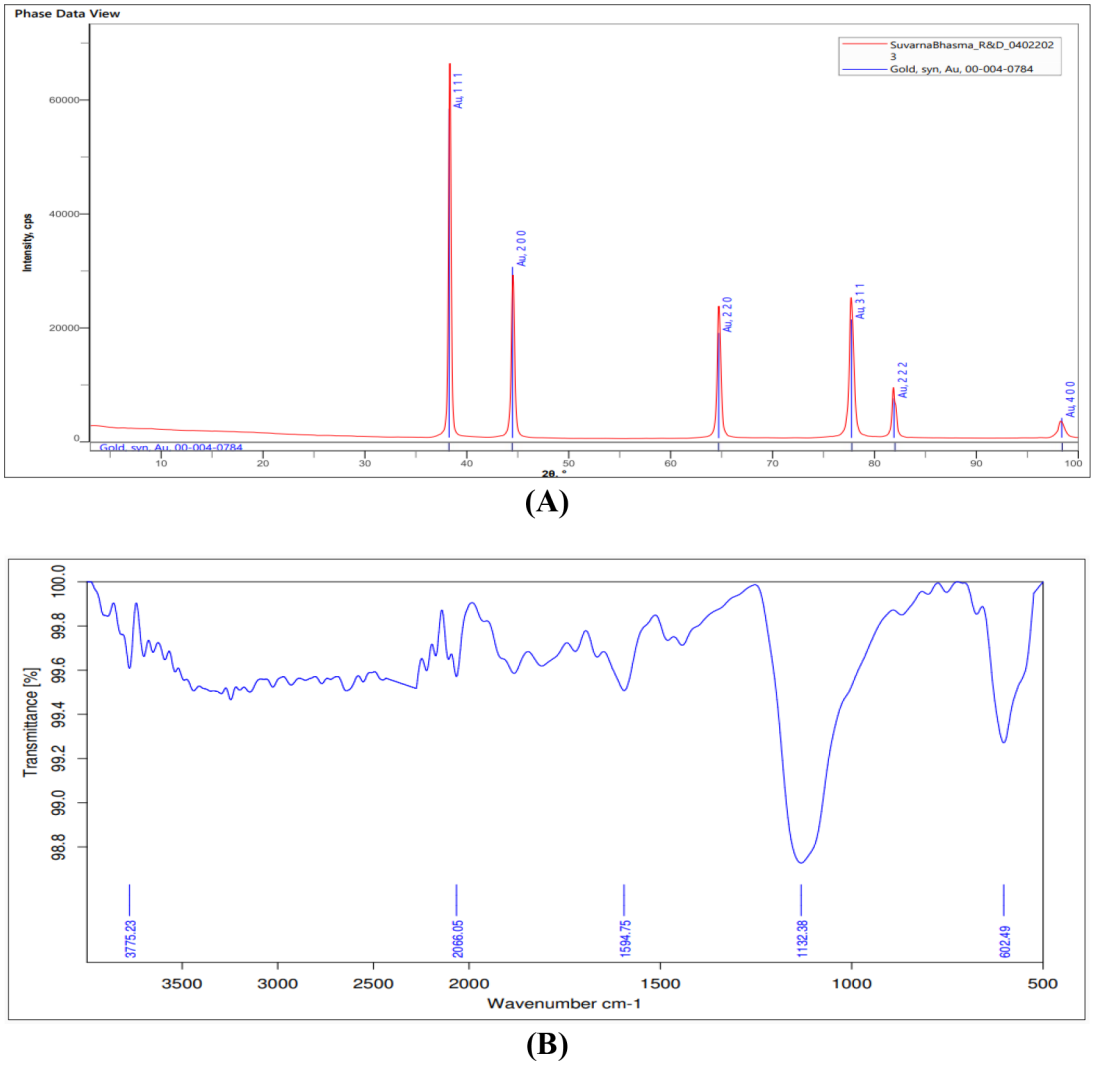


Figure 1. **(A)** XRD analysis demonstrates characteristic gold peaks, confirming crystalline nature of *Swarna Bhasma*. **(B)** FTIR spectra confirm the absence of organic residues, indicating successful incineration.

evidence of sex-specific differences in developmental survival or adult emergence.

2.12 Statistical Analysis

All experiments were conducted with three independent biological replicates per condition, and data are presented as the mean ± standard error of the mean (SEM). Graphs and statistical analyses were generated using GraphPad Prism version 5.0 (GraphPad Software, USA). Differences among multiple groups were analysed using one-way Analysis Of Variance (ANOVA), followed by Tukey's post hoc multiple-comparison test. The assumptions of normality and homogeneity of variance were evaluated before applying ANOVA. When these assumptions were not satisfied, the data were transformed or analysed using an appropriate nonparametric alternative. A two-sided pvalue < 0.05 was considered statistically significant. In the figures, groups not sharing the same letter or symbol were considered significantly different according to the specified post hoc comparison.

3. Results

3.1 SB Physicochemical Profile

The XRD pattern of SB showed distinct diffraction peaks at 2θ values of 38.2°, 44.4°, 64.6°, and 77.6° (Figure 1A). These findings confirmed that crystalline elemental gold was the predominant XRD-detectable phase in the sample[29]. The FTIR spectrum exhibited absorption bands at 3775 and 2066 cm^{-1} (Figure 1B). The band at 3775 cm^{-1} was tentatively attributed to O-H stretching associated with adsorbed moisture or surface hydroxyl groups, whereas the band at 2066 cm^{-1} remained unassigned. Apart from these bands, no prominent absorption features attributable to common organic functional groups were detected. Collectively, the findings support the presence of crystalline metallic gold with limited detectable IR-active surface constituents.

3.2 Larval Feeding Behaviour

The dye-based feeding assay showed no significant differences in dye intake between SB-treated larvae and either the untreated or vehicle-control groups (Figure 2A). These findings indicate that SB exposure did not detectably affect larval food consumption under the experimental conditions.

3.3 Developmental Outcomes and Adult Morphology

3.3.1 Developmental Timing and Larval Growth

SB exposure did not alter developmental timing, as larvae from all treatment groups reached the third-instar stage within 72 h, comparable to the untreated and vehicle controls. Larval dimensions were also comparable across all groups, with no statistically significant differences observed (Figure 2B). However, larval weight exhibited a non-linear dose-dependent response. Larvae exposed to 2.5 µg/mL SB showed significantly greater body weight (113% of the untreated control; $p<0.01$), whereas those exposed to 0.6 and 10 µg/mL SB had significantly lower body weights (70% and 87% of control, respectively; $p<0.05$) (Figure 2C). No significant differences were observed in the 1.25 or 5.0 µg/mL SB groups or in the honey-ghee vehicle-control group.

3.3.2 Pupation and Adult Emergence

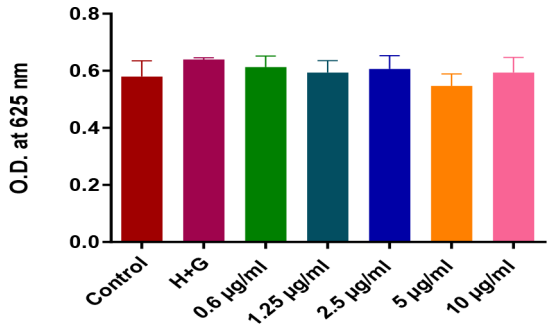
SB exposure produced only modest changes in pupation. Pupation rates were lower than the untreated control in most SB-treated groups, whereas the 1.25 µg/mL group showed a rate comparable to the control (approximately 102%). In contrast, adult emergence exhibited a biphasic concentration-dependent response (Figure 2D). Emergence was highest at 2.5 µg/mL SB, reaching 122% of the control ($p<0.001$). At higher concentrations, adult emergence declined to approximately 83% at 5 µg/mL and 52% at 10 µg/mL (both $p<0.01$). Thus, although larvae progressed to pupation, the marked reduction in adult emergence at higher SB concentrations indicates impaired pupal-to-adult development.

3.3.3 Adult Phenotype and Morphology

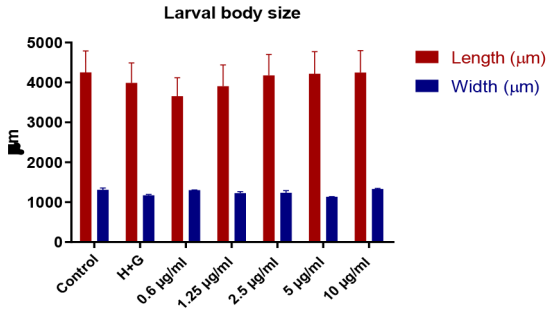
Despite the observed variations in developmental survival and larval weight, SB exposure did not produce detectable morphological abnormalities in adult flies. Microscopic examination showed normal wing size, shape, and structural organisation (Figure 3A); intact eye morphology with regular ommatidial arrangement and pigmentation (Figure 3B); and normal thoracic structure and bristle patterning (Figure 3C) across the examined groups.

3.3.4 Sex Ratio

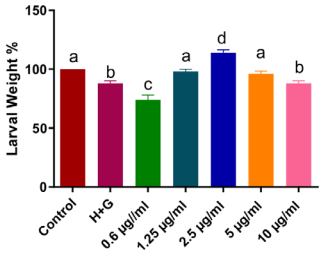
The male-to-female ratio of adult offspring was approximately 1:1 in the control group and remained



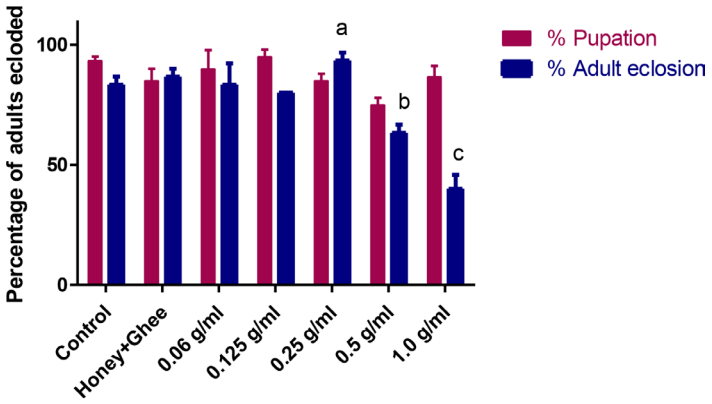
(A)



(B)

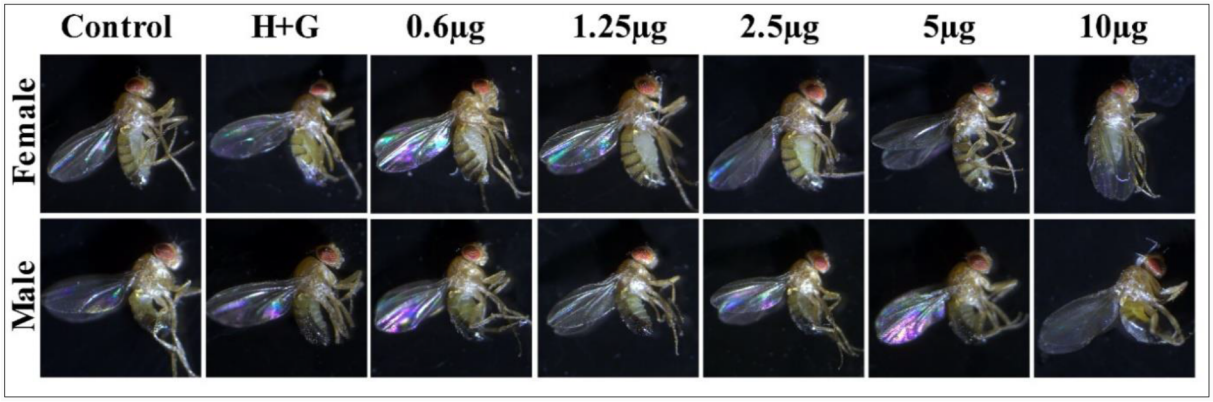


(C)

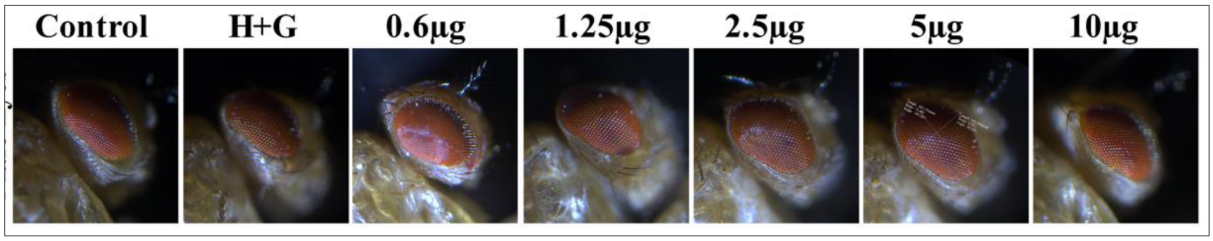


(D)

Figure 2. **(A)** Feeding behaviour remained unchanged across groups, **(B)** Larval body size (length and width) was unaffected by *Swarna Bhasma* concentration, **(C)** Larval weight increased at 2.5 µg/ml but decreased at 0.6 and 10 µg/ml, **(D)** Adult emergence peaked at 2.5 µg/ml and declined at higher concentrations, while pupation rates remained stable.



(A)



(B)

Figure 3. **(A)** Wings and scutellar bristles show no deformities across *Swarna Bhasma* concentrations, **(B)** Eyes bristles show no deformities across *Swarna Bhasma* concentrations.

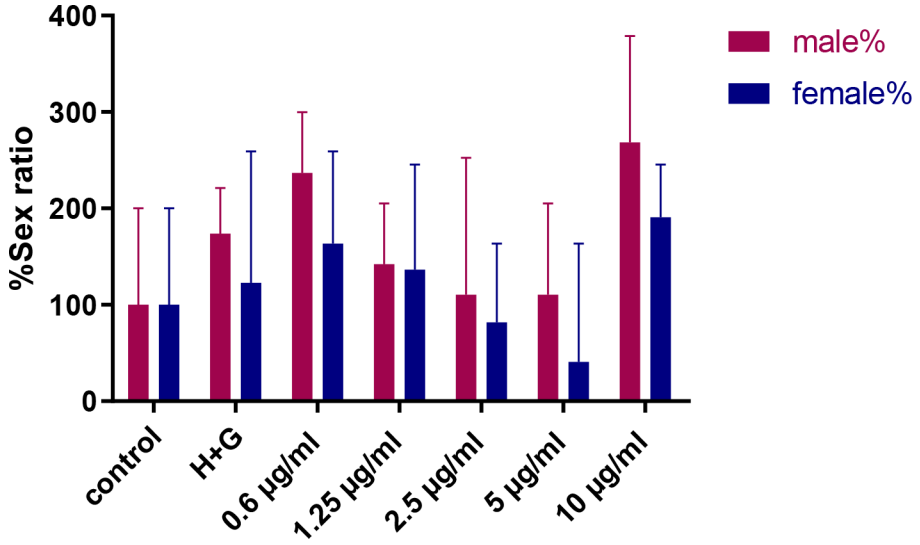


Figure S1. Sex ratio of adults emerging across treatment groups.

largely unchanged following SB exposure. A numerical predominance of males was observed at 0.6 and 1.25 µg/mL SB, whereas females predominated at 10 µg/mL. At the highest concentration, the proportion of males was significantly lower than that in the control group in the post-hoc comparison ($p<0.05$; Figure S1).

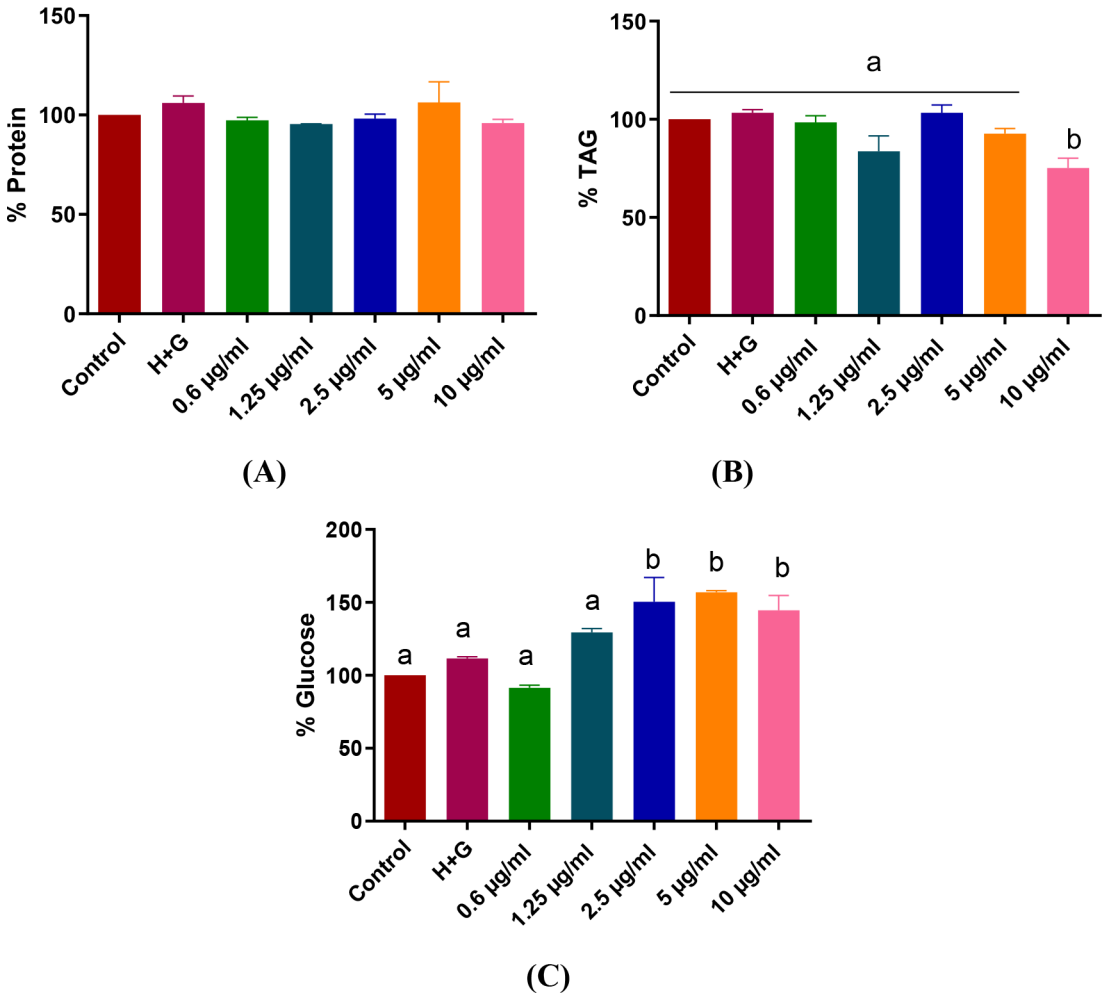


Figure 4. Dose-dependent effects of *Swarna Bhasma* on larval metabolic parameters. **(A)** Total protein concentration, **(B)** TAG, **(C)** Glucose concentration. Data are presented as mean ± SEM (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

3.4 Metabolic and Oxidative Responses

3.4.1 Macromolecule Levels (Protein, Lipid, Carbohydrate)

Total protein content remained comparable across all SB-treated and control groups (Figure 4A). TAG levels were unchanged at low and moderate SB concentrations; however, exposure to 10 µg/mL SB resulted in an approximately 25% reduction relative to the untreated control ($p<0.05$; Figure 4B). Glucose levels increased at 2.5, 5, and 10 µg/mL SB compared with the control (Figure 4C). The increase was modest at 2.5 µg/mL and more pronounced at 5 and 10 µg/mL. Given the absence of differences in larval food consumption, these changes indicate an SB-associated alteration in energy metabolism rather than an effect secondary to reduced food intake.

3.4.2 Oxidative Stress Markers

The NBT assay showed that ROS levels remained comparable to those of the controls at most SB concentrations (Figure 5A). A significant 37% increase in ROS was observed at 5 µg/mL SB ($p<0.05$), whereas ROS levels at 10 µg/mL did not differ significantly from the control. Nitrite levels, used as an index of nitric oxide production, were significantly reduced by approximately 34-38% at 0.6 and 10 µg/mL SB ($p<0.01$; Figure 5B). Intermediate SB concentrations did not significantly affect nitrite levels. Collectively, these findings demonstrate concentration-specific and non-linear effects of SB on oxidative and nitrosative status.

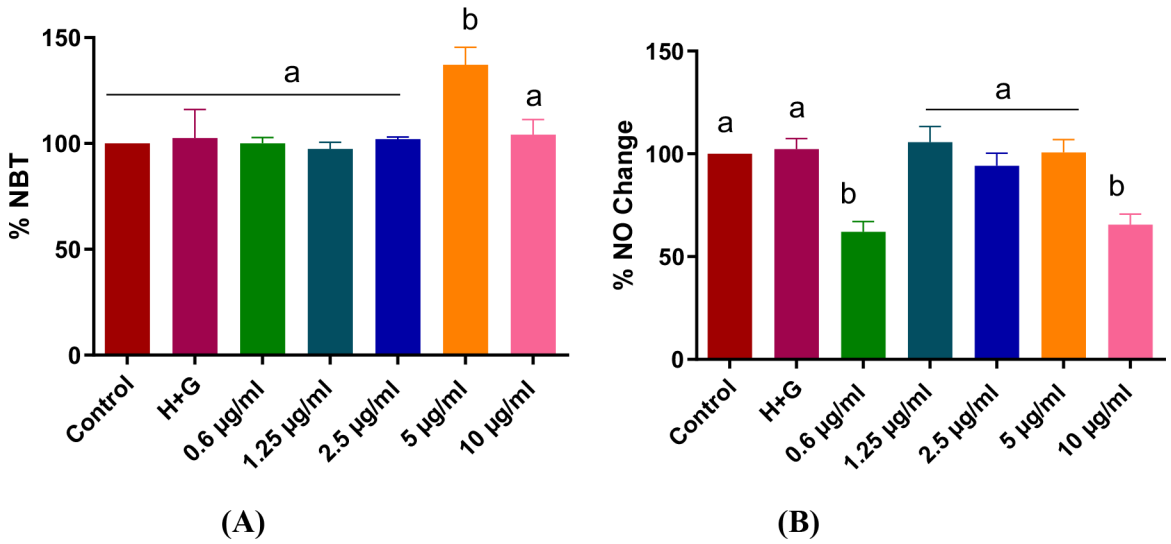


Figure 5. Dose-dependent effects of *Swarna Bhasma* on oxidative stress markers. **(A)** Reactive Oxygen Species (ROS) quantified using the Nitroblue Tetrazolium (NBT) reduction assay, **(B)** Nitric Oxide (NO) levels determined using the Griess reaction. Data are expressed as mean ± SEM (n = 3). Statistical significance was analysed using one-way ANOVA followed by Tukey's post hoc test.

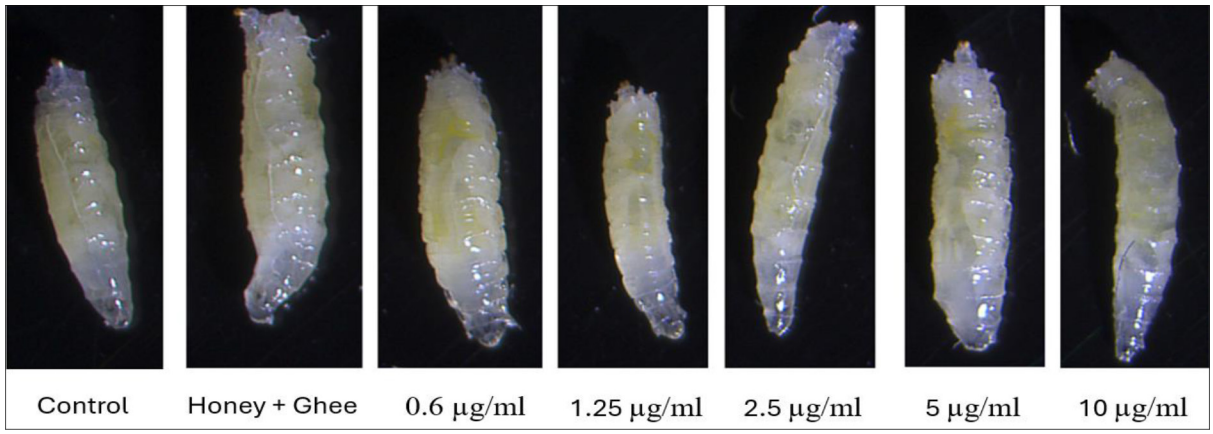


Figure S2. Trypan blue staining revealed no intestinal damage from *Swarna Bhasma* treatment.

3.5 Neurobehavioural and Locomotor Activity

3.5.1 Larval Crawling Behaviour

SB exposure produced a significant biphasic effect on larval locomotion ($p<0.001$). Larvae exposed to 0.6 and 1.25 µg/mL SB exhibited increased crawling activity and speed compared with the controls. Locomotor performance at 2.5 µg/mL was comparable to that of the control groups, whereas larvae exposed to 5 and 10 µg/mL showed reduced movement and crawling speed (Figure 6). These findings demonstrate locomotor stimulation at lower SB concentrations and impairment at higher concentrations.

3.5.2 Adult Climbing Performance

Adult negative geotaxis performance exhibited a biphasic concentration-dependent response to SB. Flies exposed to 2.5 µg/mL SB showed the greatest climbing ability, with a significantly higher proportion crossing the 6 cm mark within 10 s than in the control group ($p<0.01$). In contrast, flies exposed to 5 and 10 µg/mL SB exhibited significantly reduced climbing performance ($p<0.001$),

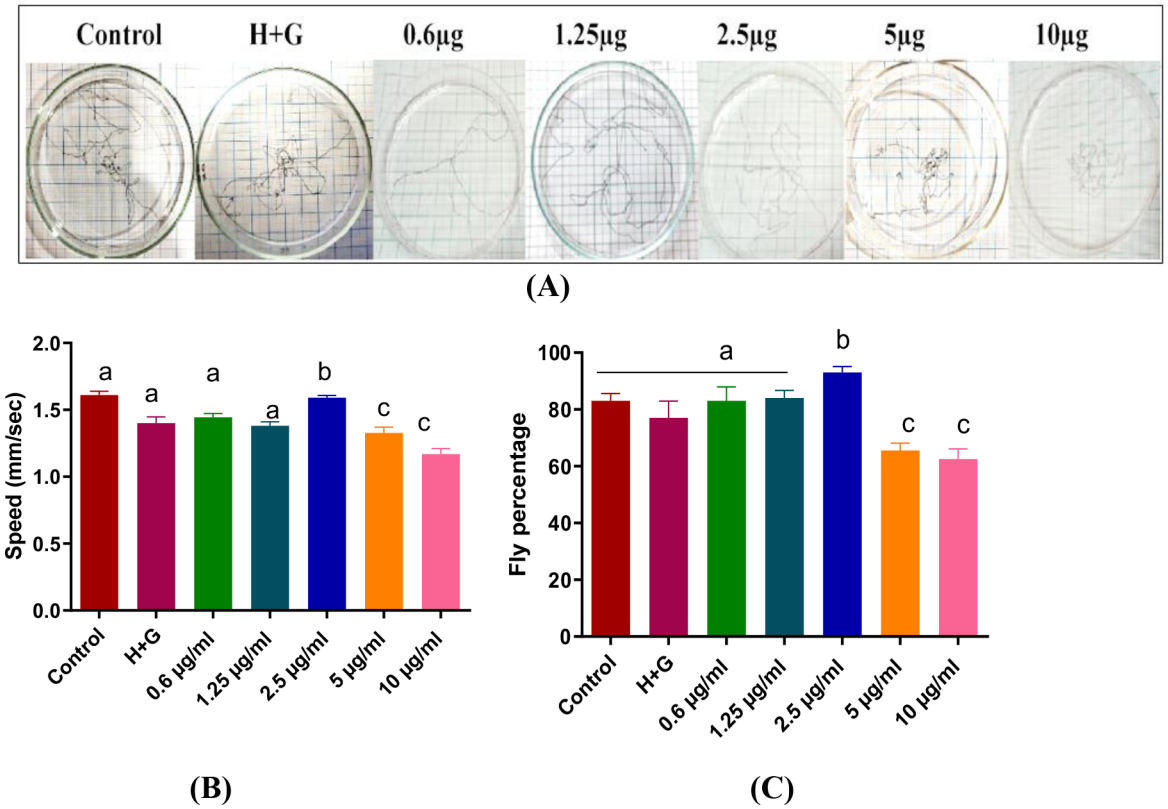


Figure 6. Behavioural responses following developmental exposure to *Swarna Bhasma*. **(A)** Representative larval crawling tracks on agar, **(B)** Mean larval crawling speed (mm/s), **(C)** Adult negative geotaxis performance expressed as the percentage of flies crossing the 6 cm mark within 10 s. Data are presented as mean ± SEM (n = 3 independent experiments). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test (*p<0.05, **p<0.01, *p<0.001 versus control).

demonstrating impaired locomotor coordination at higher SB concentrations.

3.6 Tissue Integrity and Survival

Trypan Blue staining revealed no detectable intestinal epithelial damage in larvae exposed to any tested concentration of SB. Similar to the control groups, SB-treated larvae showed no blue staining of the intestinal tissue, indicating the absence of detectable epithelial cell death or disruption of gut-barrier integrity. Notably, no mucosal damage was observed even at the highest concentration of 10 µg/mL SB (Figure S2).

4. Discussion

This study investigated the dose-dependent neurobehavioural and toxicological effects of *Swarna Bhasma* (SB), a traditionally processed gold-based Ayurvedic nanomedicine, using *Drosophila melanogaster* as an in vivo whole-organism model. To our knowledge, this is the first study to evaluate SB with its classical adjuvants—honey and ghee—thereby experimentally examining its traditional mode of administration. Assessment of developmental, behavioural, metabolic, redox, morphological, and intestinal outcomes revealed a nonlinear dose-response relationship. The findings identify 2.5 µg/mL as the concentration providing the most favourable balance between neurobehavioural activity and tolerability, while higher concentrations produced functional and biochemical alterations.

Larval crawling increased at lower SB concentrations but declined at higher concentrations, whereas adult negative geotaxis improved most at 2.5 µg/mL and was impaired at 5-10 µg/mL. Because these assays reflect neuromuscular function, sensorimotor coordination, and nervous-system performance in *Drosophila*, the results indicate that SB exerted a biologically relevant neurobehavioural effect rather than acting as an inert

dietary component. This biphasic pattern is consistent with a hormetic-like response, in which low-to-moderate exposure may activate adaptive pathways associated with cellular stress resistance, mitochondrial function, and neuronal signaling, whereas higher exposure may exceed the capacity of these protective mechanisms.[7,8] However, the molecular basis of this response was not directly examined. The reduced proportion of flies crossing the specified height at higher concentrations indicates impaired locomotor efficiency despite the absence of gross morphological abnormalities.

Although pupation was largely unaffected, adult emergence improved at an intermediate concentration but declined significantly at concentrations ≥5.0 µg/mL. This finding suggests that the pupal-to-adult transition was more sensitive to SB exposure than the earlier developmental stage. Metamorphosis is metabolically demanding and involves extensive tissue remodeling, organogenesis, and neuromuscular maturation. Increased energetic demand and redox imbalance during this period may therefore increase susceptibility to higher SB concentrations, although this interpretation requires mechanistic confirmation.

The feeding assay showed no significant differences in dye-tracer consumption among the groups, indicating that the developmental and behavioural effects were unlikely to result from feeding aversion, reduced palatability, or starvation. This is an important methodological strength because reduced food intake can confound dietary nanomaterial studies. The consumption-excretion approach is an established method for estimating ingestion in *Drosophila* and reducing uncertainty regarding dietary exposure[9]. Nevertheless, comparable consumption confirms similarity in external exposure but does not establish equivalent absorption or internal tissue concentrations.

Swarna Bhasma selectively altered carbohydrate and lipid metabolism without significantly affecting total protein content. Glucose levels increased at 2.5-10 µg/mL, whereas TAG levels decreased only at 10 µg/mL. These changes suggest altered energy allocation, with disruption of glucose homeostasis and mobilisation of lipid reserves becoming more evident at higher exposure levels. The preservation of total protein indicates that overall tissue protein homeostasis was not substantially affected. Similar alterations in carbohydrate homeostasis have been reported following gold nanoparticle ingestion in *Drosophila*[13]. The concurrent elevation of glucose, depletion of lipid reserves, and impairment of climbing at higher concentrations may indicate metabolic strain contributing to locomotor dysfunction. Measurements of insulin signalling, mitochondrial activity, ATP production, and energy expenditure are required to confirm this relationship.

Among the oxidative-stress parameters, NBT-reactive species increased significantly only at 5 µg/mL, whereas nitric oxide levels decreased at both the lowest and highest concentrations. This nonparallel response suggests differential modulation of oxidative and nitrosative pathways rather than a uniform increase in redox stress. The response at 5 µg/mL may represent a threshold at which superoxide-related activity becomes detectable. The absence of a corresponding increase at 10 µg/mL could reflect a nonlinear response, compensatory antioxidant activity, reduced metabolic activity, selective survival, or assay sensitivity. Additional evaluation of antioxidant enzymes, glutathione status, lipid peroxidation, and mitochondrial function is needed to clarify the redox mechanisms involved[30].

The adverse effects observed at higher SB concentrations were predominantly functional and biochemical rather than grossly structural. Adult flies showed no detectable abnormalities in their wings, eyes, or thoracic bristles, and trypan blue staining revealed no overt intestinal epithelial injury, even at 10 µg/mL. Engineered metallic nanoparticles, including gold nanoparticles, have been associated with abnormal phenotypes in *Drosophila* and developmental disruption in zebrafish[9,12]. In contrast, SB did not produce detectable morphological or intestinal structural damage under the present conditions. Nevertheless, differences in composition, particle characteristics, exposure methods, and dose preclude direct equivalence between SB and engineered gold nanoparticles.

The absence of trypan-blue-positive intestinal injury supports the conclusion that SB did not cause overt epithelial toxicity. However, trypan blue primarily detects substantial membrane damage or cell death and cannot identify subtle alterations in barrier permeability, epithelial transport, luminal aggregation, microbiome composition, or subcellular stress. Therefore, these findings demonstrate the absence of detectable gross intestinal injury but do not exclude more subtle physiological effects or establish the absorption and tissue distribution of SB.

A major strength of this study was its whole-organism evaluation of developmental, neurobehavioural,

metabolic, redox, morphological, and intestinal outcomes. Untreated and vehicle controls helped distinguish SB-associated effects from those of the honey-ghee-DMSO vehicle. Translationally, the findings identify a preliminary biological safety window separating favourable neurobehavioural effects from higher-dose functional toxicity. This dose-response profile can guide the selection of exposure ranges for subsequent vertebrate studies; however, the concentration of 2.5 µg/mL cannot be directly extrapolated to mammals without pharmacokinetic and dose-conversion investigations.

The study primarily assessed organism-level toxicity and behavioural outcomes without measuring gold accumulation, biodistribution, mitochondrial bioenergetics, neurotransmitters, antioxidant enzymes, inflammatory signalling, or neuronal histopathology. Therefore, although the findings indicate a favourable safety profile within a defined experimental range, the mechanisms underlying the hormetic-like response remain uncertain. Future pharmacokinetic, biochemical, histological, and transcriptomic studies are required to clarify these mechanisms and support the translational evaluation of SB.

5. Conclusion

Swarna Bhasma administered with honey and ghee produced a dose-dependent biphasic response in *Drosophila melanogaster*. The 2.5 µg/mL concentration showed the most favourable biological profile, with improved adult emergence and locomotor performance, whereas 5-10 µg/mL caused locomotor impairment and metabolic and redox alterations. The absence of gross morphological abnormalities or intestinal epithelial damage indicates that the observed adverse effects were primarily functional and biochemical rather than structural. These findings identify a preliminary experimental safety window; however, mechanistic, biodistribution, pharmacokinetic, and mammalian studies are required before clinical extrapolation.

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Author Contribution

SD, AS: Conceptualization, Manuscript writing
KC, SP: Review and editing of manuscript

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