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Protective Role of Aframomum melegueta Against Arsenic-Induced Oxidative and Neurobehavioral Toxicity in Drosophila melanogaster

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ABSTRACT:

Purpose: *This study investigated the protective effects of the ethanol extract of Aframomum melegueta against arsenic-induced oxidative stress, biochemical alterations, and neurobehavioral dysfunctions in Drosophila melanogaster. The aim was to determine whether the Extract could mitigate arsenic-mediated lipid peroxidation, antioxidant suppression, and locomotor impairment, while improving survival outcomes.*

Methodology: *Wild-type Drosophila melanogaster were divided into four groups: control, arsenic-treated, extract-treated, and combined treatment. Arsenic toxicity was induced using sodium arsenite, and the plant extract was administered through dietary inclusion. Biochemical assays (MDA, catalase, GST, thiol, protein levels), survival studies, negative geotaxis, and larval locomotion tests were performed. Data were analyzed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).*

Findings: *Arsenic exposure significantly increased MDA and reduced catalase activity and thiol levels, indicating oxidative stress. Aframomum melegueta significantly reversed these alterations, reducing MDA and restoring catalase and thiol concentrations. GST activity increased significantly in the combined treatment. Behaviourally, arsenic impaired climbing ability and locomotor activity, while the Extract improved locomotor distance and demonstrated significant antioxidative effects and modest improvement in selected neurobehavioral parameters. Survival rates declined sharply in arsenic-treated groups but improved moderately with extract supplementation.*

Research Implications/Limitations: *The study provides preliminary evidence that Aframomum melegueta exerts antioxidative effects and modest neurobehavioral protection against arsenic-induced toxicity in Drosophila melanogaster. However, the absence of mechanistic analyses and reliance on an invertebrate model limit direct translational interpretation.*

Practical Implications: *The findings suggest that Aframomum melegueta may represent a potential source of bioactive compounds with antioxidative properties, warranting further mechanistic*

and translational investigation. Its antioxidant properties highlight its potential for incorporation into nutraceutical formulations. However, optimal dosing, long-term safety, and clinical applicability remain to be established.

KEYWORDS:

Aframomum melegueta, Antioxidants, Arsenic toxicity, Drosophila melanogaster, Lipid peroxidation, Oxidative stress, Survival assay.

1. Introduction

Arsenic, a naturally occurring metalloid, presents a considerable health hazard when found in the environment at high concentrations [1]. Arsenic is present in groundwater, soil, and air as a result of both natural processes and human activities, including mining, industrial waste discharge, and the application of arsenic-laden pesticides [2]. Prolonged exposure to arsenic, particularly via drinking water, has been linked to several detrimental health effects, including cardiovascular illnesses, dermal lesions, and multiple cancer types [3]. Arsenic enters the human body mainly by eating or inhalation, and upon absorption, it is metabolically converted into highly hazardous metabolites, including trivalent and pentavalent arsenic compounds [4]. These substances trigger a series of detrimental consequences at the cellular level, including oxidative stress, DNA damage, and disruption of essential metabolic processes [5].

Extended arsenic exposure has been widely associated with oxidative stress, cellular dysfunction, and neurological impairments across experimental models [6]. Oxidative stress is crucial in the onset and advancement of several cancer types, including arsenic-induced lung cancer [7]. Overproduction of reactive oxygen species (ROS) may lead to significant cellular damage through the oxidation of proteins, lipids, and DNA. Consequently, they may serve as a primary catalyst for carcinogenesis by fostering genetic instability, cell death, and chronic inflammation, all of which facilitate tumor development [8]. Scientific evidence has shown that arsenic exposure markedly increases ROS generation in arsenic-induced cancer, surpassing the body's antioxidant defense mechanisms [9]. This may result in lipid peroxidation, protein oxidation, and DNA strand breaks, all of which facilitate the onset and advancement of cancer [10]. *Aframomum melegueta*, referred to as grains of paradise, is a tropical plant in the Zingiberaceae family. The seeds possess a spicy, peppery flavor and are abundant in bioactive components such as gingerols, paradols, shogaols, and essential oils [11]. These compounds possess antioxidant, anti-inflammatory, and anticancer effects. In traditional and contemporary West African cuisines, *Aframomum melegueta* serves as a principal flavoring agent in soups, jollof rice, and pepper soup [12,13]. The seeds are typically subjected to maceration to facilitate the liberation of volatile aromatic constituents [14]. In Southwestern Nigeria, the seed of *Aframomum melegueta* is well-reputed in ceremonial, ritualistic, and sociocultural practices, where it may function as a masticatory or be exchanged as an emblem of hospitality and benediction [15]. The antioxidant efficacy of *Aframomum melegueta* is mostly ascribed to its elevated phenolic and flavonoid concentrations, which facilitate the neutralization of free radicals and safeguard cells from oxidative harm [16]. This study was designed as an exploratory investigation to evaluate whether the ethanol extract of *Aframomum melegueta* can attenuate arsenic-induced oxidative stress, biochemical disruptions, and neurobehavioral impairments in *Drosophila melanogaster*. We hypothesized that the Extract would mitigate arsenic toxicity primarily through modulation of oxidative stress markers and antioxidant defense enzymes. While specific molecular signaling pathways were not directly examined, the findings are intended to provide a foundation for future mechanistic studies.

2. MATERIALS

Drosophila melanogaster, Spatula, Conical flask (200ml and 1000ml), Trays, Vials, Foam (cork), Eppendorf tubes, Test tubes, Rack, Tips, Small paint brush, Refrigerator, Petri dish, Counting pad, Jars, Gloves, Animal feed, Distilled water, Centrifuge, Sensitive scale, Incubator, Hot plate, Homogenizer, Arsenic compound (26061340191), *Aframomum melegueta* seeds, Ethanol, Anesthetic CO_2.

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3. METHODS

EXPERIMENTAL DESIGN

Drosophila melanogaster was obtained and cultured in the Eureka Research Laboratory, Department of Anatomy, at Babcock University. The flies were maintained at a temperature of 25℃. After the breeding phase, the flies were divided into four treatment groups as follows:

TABLE 1: EXPERIMENTAL DESIGN

Group	Treatment	Purpose
Control	Basal diet	Serve as a baseline for comparison with other experimental groups.
Arsenic-Treated	0.005g/10g of diet	Assess the impact of arsenic exposure on oxidative stress markers and antioxidant defenses.
Plant extract	0.015g/10g of diet	Observe effects of the Extract on normal cellular processes and oxidative stress in the absence of arsenic.
Combined Treatment Group	0.005g+ 0.015g/10g of diet	Investigate the effects of the Extract against arsenic-induced oxidative stress and toxicity conditions.

Each experimental group consisted of five independent vials, with fifty flies per vial (total n = 250 flies per group). Behavioral assays were performed using randomly selected flies or larvae from each vial to ensure representative sampling. A single-dose experimental framework was employed to evaluate baseline toxic and protective effects prior to dose–response optimization.

ARSENIC EXPOSURE

Arsenic Compound: Sodium arsenite ($NaAsO_2$) was used to induce oxidative stress and lung cancer-like phenotypes in *Drosophila melanogaster*.

IDENTIFICATION AND PREPARATION OF PLANT

Aframomum melegueta was obtained from Ilishan-Remo, Ogun State, Nigeria. Fresh seeds of *A. melegueta* were air-dried (16 °C), pulverized, and soaked in absolute ethanol for 72 hours, then sieved in order to remove any non-polar compounds from the Extract. and stored in a jar.

PHYTOCHEMICAL CHARACTERIZATION BY HPLC

High-performance liquid chromatography (HPLC) analysis was conducted to characterize the phytochemical constituents of the ethanol extract of *Aframomum melegueta*. The Extract was prepared according to standard chromatographic procedures and analyzed using an HPLC system equipped with a UV–Vis detector. Chromatograms were recorded at multiple wavelengths to detect phenolic and related bioactive compounds. The resulting chromatographic profile was used to generate a chemical fingerprint of the Extract employed in this study.

DROSOPHILA MELANOGASTER STOCK AND CULTURE

The wild D. melanogaster was obtained from the Eureka *Drosophila* Laboratory, in the Department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ogun State, Nigeria. *D. Melanogaster* cultured on a corn meal medium containing corn meal, yeast, sugar, agar-agar and nipagin mixed with ethanol at a constant temperature and humidity (21-25°C; 60-70% relative humidity) under 12hours dark/light cycle conditions at the Eureka *drosophila* laboratory, in the department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ogun State, Nigeria.

MEAL PREPARATION

I. 1000ml of water was added to a pot and allowed to boil on an electric cooker.

II. A sensitive scale was used to measure 26g of yeast, and 25g of sugar was added to the boiling water, stirring this mixture for 5 minutes.

III. Using the sensitive scale again, 13g of agar-agar was measured and added gently to the boiling mixture inside the pot, and then stirred for 5 minutes.

IV. 200ml of distilled water was used to mix 90g of cornmeal in a mixing bowl. After it was mixed properly, it was added to the still-boiling mixture and stirred for 5 minutes.

V. 1 Cap of *Aframomum melegueta* and 1 cap of arsenic dissolved in ethanol were mixed with 5g of Nipagin in a petri-dish, and this was carefully combined.

VI. The Cornmeal mixture was brought down from the electric cooker and allowed to cool for some minutes.

VII. The Nipagin mixture was added to the Cornmeal mixture after being cooled and stirred.

TABLE 2: TABULAR REPRESENTATION OF MEAL PREPARATION

S/N	INGREDIENTS	FULL MEAL
1.	Distilled water	1000ml
2.	Corn meal	90g
3.	Yeast	26g
4.	Nipagin	5g
5.	Agar agar	13g
6.	Sugar	25g

DOSAGE AND ADMINISTRATION

The ethanol crude extract was solubilized in distilled water and integrated into the diet of *Drosophila melanogaster*. The arsenic dose (0.005 g sodium arsenite per 10 g of diet) was selected based on previously reported *Drosophila melanogaster* studies demonstrating consistent induction of oxidative stress and neurobehavioral impairment without causing excessive early mortality, thereby allowing meaningful biochemical and behavioral assessments.

The dose of *Aframomum melegueta* extract (0.015 g per 10 g of diet) was selected following preliminary tolerability observations, which indicated no overt toxicity or adverse behavioral effects in flies, and in alignment with doses reported for antioxidant-rich plant extracts in *Drosophila* and related in vivo models. This single-dose approach was adopted to establish initial efficacy under controlled exposure conditions.

Arsenic was delivered by dietary consumption. The exposure period was extended for 7 days, providing adequate Time for the manifestation of oxidative stress indicators. At the conclusion of the seventh day, a specific group of flies was allocated for biochemical and behavioral experiments. The remaining cohort of flies had to undergo a survival test for an additional 14 days, culminating in a total duration of 21 days.

BIOCHEMICAL ASSAY

Malondialdehyde (MDA)

The MDA concentration was analyzed as previously described by [17].

Total Thiol Content:

Total thiol concentration was described based on the method of [18].

Glutathione S-transferase

The GST activity was measured spectrophotometrically based on the method of [19]. The activity of GSH was expressed in μmol/mg protein.

Catalase Activity

Catalase activity was assessed by introducing H_2O_2 to the sample and observing its breakdown by the measurement of absorbance reduction at 240 nm. Catalase activity was quantified in units per milligram of protein [20].

Total Protein Content

Bradford Assay

The total protein content was quantified using the Bradford technique, which depended on the interaction of Coomassie Brilliant Blue dye with proteins, producing a colorimetric change measurable at 595 nm [21].

SURVIVAL TEST

The survival test was carried out to monitor the number of flies that survive for 21 days following exposure to arsenic and the plant extract. Each day, the number of active flies was counted to see the effects of the plant extract. The vials were cleaned properly, and all the dead flies were removed after 7 days consecutively for 21 days.

BEHAVIORAL ASSAY

NEGATIVE GEOTAXIS ASSAY

Negative geotaxis assay, also known as the climbing assay, was carried out to determine the locomotor activity of the flies. To carry out this

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experiment, 10 flies from the control and treated group were anesthetized and placed in labeled vials marked at 6cm. The bottom of the vials was gently tapped, and then allowed to ascend. The number of flies in each vial that ascended to the 6cm level in 10 seconds was counted, and the numbers were recorded and used to determine the climbing activity.

LARVA CRAWLING ASSAY

From each group, ten (10) third instar larvae were collected with a damp (with sucrose) paint brush and placed on a slightly wet (with sucrose) petri dish to aid the movement of the larvae. Records of larva movements were recorded for 10s, after which the videos were analyzed. Larvae's distance traveled, and number of contractions were noted down within the 10s. The speed and contraction ratio were calculated using the following formula:

Speed (s) = Distance traveled by the lava / Time taken

Contraction ratio = Distance traveled by the larva/number of contractions

STATISTICAL ANALYSIS

The data from the biochemical assays were analyzed using one-way analysis of variance (ANOVA) followed by post hoc comparisons with Tukey's test. This allowed for the comparison of means across different treatment groups and determined whether the differences were statistically significant ($p < 0.05$). Data are presented as mean ± SEM. Behavioral measurements were obtained from randomly selected flies drawn from five independent vials per group (50 flies per vial).

4. RESULTS

HPLC Phytochemical Profile of Aframomum melegueta Extract

HPLC analysis of the ethanol extract of *Aframomum melegueta* revealed the presence of multiple phytochemical constituents, evidenced by distinct peaks observed at different retention times and detection wavelengths (Figs. 1a and 1b). The chromatographic fingerprint indicates a complex mixture of phenolic and related bioactive compounds, consistent with previously reported antioxidant constituents of *Aframomum melegueta*. These phytochemicals likely contribute to the observed antioxidative and neurobehavioral effects in arsenic-exposed *Drosophila melanogaster*.

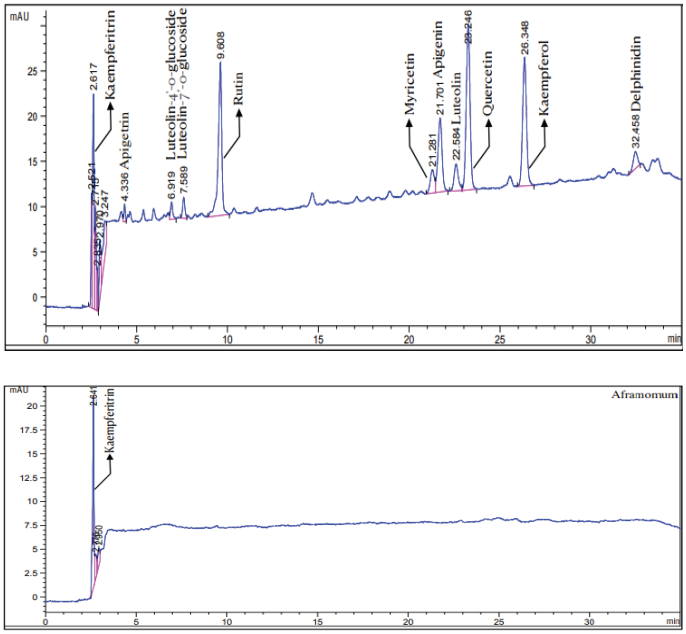

Figure 1. HPLC profiles of A. melegueta ethanol extract. (A) Separation of compounds at a fixed wavelength. (B) Detection across multiple wavelengths, confirming the presence of diverse phytochemicals with unique spectral signatures.

Legend: The results demonstrate the complex phytochemical composition of *A. melegueta*. Key bioactive compounds are indicated by numbered peaks (1, 2, 3...), corresponding to metabolites identified or characterized in subsequent analyses.

Effect of Arsenic and Aframomum melegueta on Survival Rate in Drosophila melanogaster

The survival assay revealed distinct mortality patterns across treatment groups over 21 days. Control flies maintained 100% survival until day 3, gradually declining to 78-90% by day 21. Arsenic-exposed flies showed accelerated mortality, dropping to 56-60% survival by day 21, with particularly steep declines between days 4-7 (100% to 74-84%). The *Aframomum melegueta* group exhibited intermediate survival (62-88% at day 21), while the combined treatment group (arsenic + Aframomum) demonstrated the most significant protection, maintaining 54-84% survival - comparable to arsenic-alone mortality rates but with less severe early-stage decline. These patterns suggest that while Aframomum alone provided modest protection against natural aging-

related mortality, its combination with arsenic did not prevent arsenic›s acute lethal effects, though it may have mitigated some late-stage toxicity. The differential survival trajectories highlight the time-dependent nature of arsenic toxicity and potential protective mechanisms of Aframomum that may require longer exposure periods to manifest fully in survival outcomes (Fig. 2).

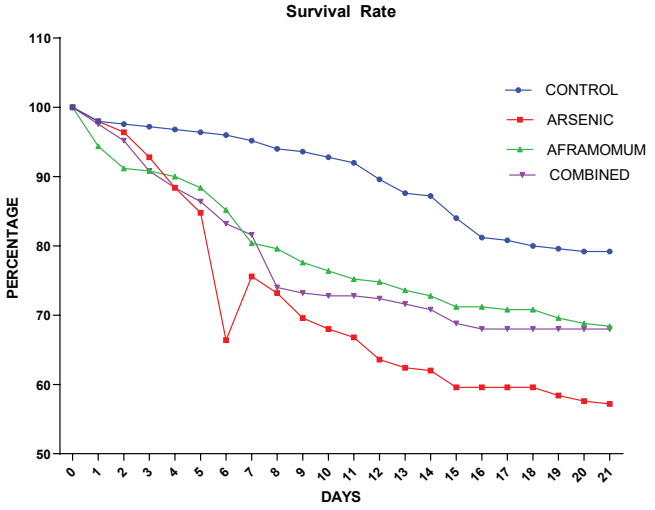

Figure 2: Effect of Aframomum melegueta extract on the survival rate of Drosophila melanogaster exposed to arsenic toxicity.

Survival percentages of flies were monitored over 21 days across treatment groups. Arsenic exposure reduced survival significantly, while *Aframomum melegueta* supplementation partially restored lifespan and mitigated late-phase mortality. Values represent mean ± SEM.

Effect of Arsenic and Aframomum melegueta on Negative Geotaxis Behavior in Drosophila melanogaster

ANOVA indicated moderate treatment effects on climbing behavior, with Tukey's tests yielding mixed significance (Fig. 3). Arsenic exposure significantly impaired geotactic performance relative to control (p = 0.0168), suggesting neurobehavioral dysfunction due to oxidative damage. *A. melegueta* alone did not differ significantly from control (p = 0.9020) or arsenic (p = 0.6793). The combined treatment showed partial improvement but did not reach statistical significance (p = 0.0628–0.9729). Although *Aframomum melegueta* treatment showed a tendency toward improved climbing performance, this effect did not reach statistical significance, indicating only partial and variable behavioral modulation.

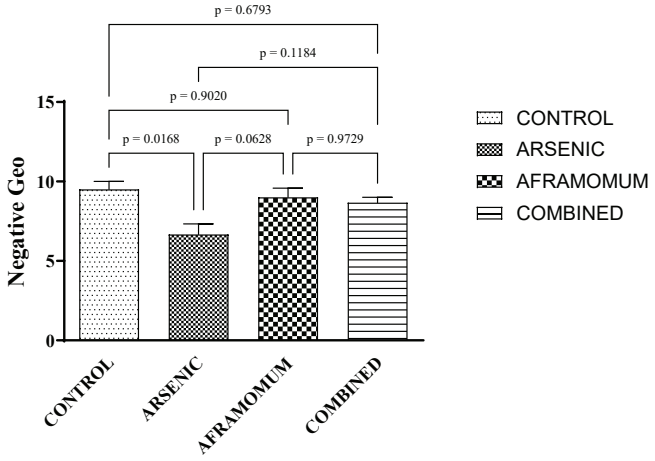

Figure 3: Effect of Aframomum melegueta extract on negative geotaxis behavior in arsenic-treated Drosophila melanogaster.

Effect of Arsenic and Aframomum melegueta on Contraction Parameters in Drosophila melanogaster

While overall ANOVA indicated treatment-dependent differences (p = 0.0408), pairwise comparisons revealed that improvements associated with *Aframomum melegueta* did not reach statistical significance, suggesting modest functional trends rather than robust effects. Tukey's test showed that arsenic exposure caused a reduction compared to control, while *Aframomum* supplementation alone or in combination partially improved contraction amplitude (Fig. 4), though these differences were not significant (p = 0.1403–0.9772). The significant ANOVA suggests at least one group differed, likely due to arsenic's depressive effect on muscular contraction, with *Aframomum melegueta* exhibiting mild protective trends against this impairment.

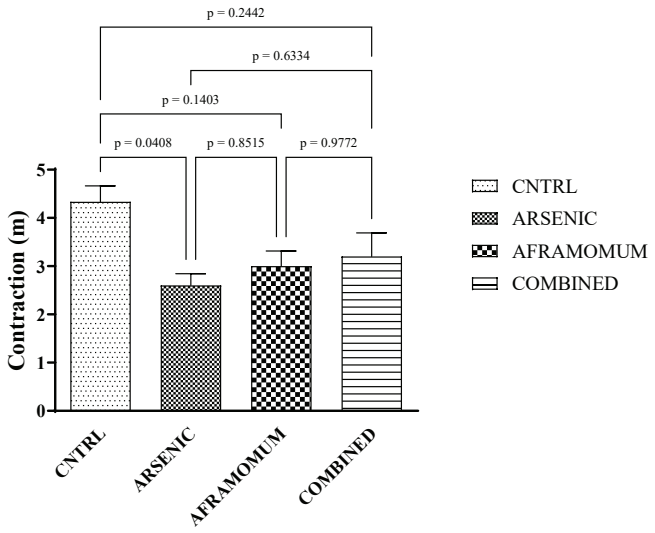

Figure 4: Effect of Aframomum melegueta extract on contraction movement in Drosophila melanogaster exposed to arsenic toxicity

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Effect of Arsenic and Aframomum melegueta on Locomotor Distance in Drosophila melanogaster

ANOVA results approached significance (p = 0.0515) with post hoc Tukey's tests indicating that *Aframomum melegueta* treatment significantly increased locomotor distance compared to arsenic exposure (p = 0.0280), indicating partial functional recovery. Control and combined groups did not differ significantly (p = 0.3973–0.9540; Fig. 5). Thus, arsenic reduced movement activity, reflecting motor impairment, whereas *Aframomum* ameliorated this reduction, highlighting its neuroprotective effect on locomotion.

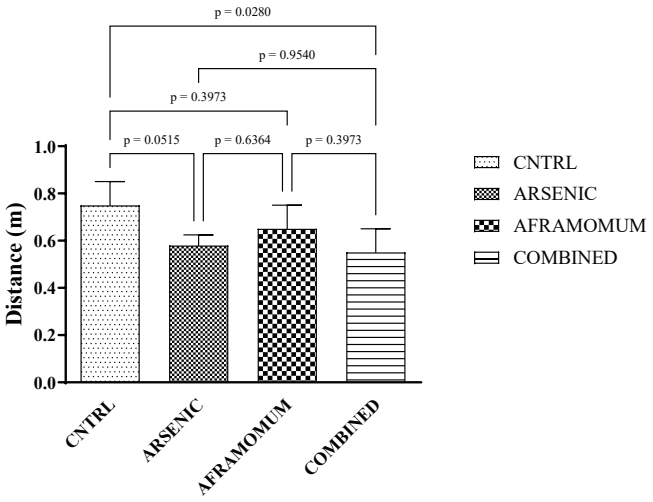

Figure 5: Effect of Aframomum melegueta extract on locomotor distance in arsenic-exposed Drosophila melanogaster

Effect of Arsenic and Aframomum melegueta on Locomotor Speed in Drosophila melanogaster

Despite a marginal overall ANOVA effect (p = 0.0400), post hoc analyses revealed no statistically significant differences between treatment groups, indicating that locomotor speed was not robustly affected (p = 0.4409–> 0.9999; Fig. 6). This suggests that while arsenic may have exerted subtle effects on movement velocity, these were not large enough to produce distinct post hoc group differences. Therefore, *Aframomum melegueta* did not significantly alter locomotor speed, though slight trends toward improvement were observed.

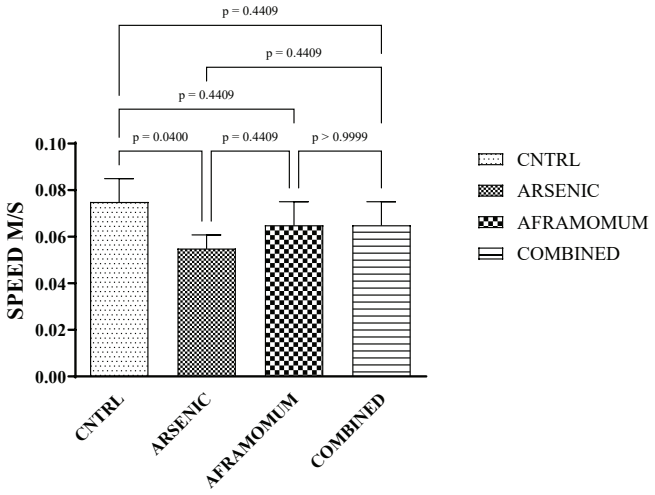

Figure 6: Effect of Aframomum melegueta extract on locomotor speed in Drosophila melanogaster exposed to arsenic toxicity

Effect of Arsenic and Aframomum melegueta on Catalase Activity in Drosophila melanogaster

ANOVA revealed a significant treatment effect on catalase activity (p = 0.0008). Post hoc Tukey's test showed that arsenic exposure caused a significant reduction in catalase levels compared with the control (p = 0.0236), indicating oxidative stress-induced enzyme inhibition (Fig. 7). Treatment with *Aframomum melegueta* significantly increased catalase activity relative to the arsenic group (p = 0.0005), while the combined treatment (arsenic + *Aframomum*) also showed a significant increase over arsenic exposure alone (p = 0.0070). These results suggest that *Aframomum melegueta* mitigated arsenic-induced oxidative suppression of catalase, restoring antioxidant capacity in *Drosophila melanogaster*.

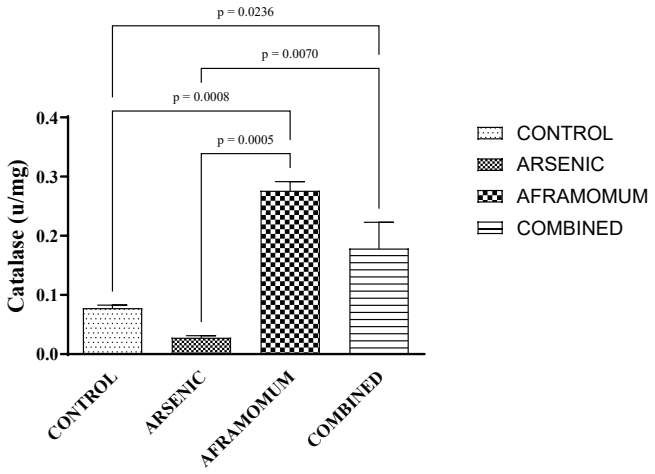

Figure 7: Effect of Aframomum melegueta extract on catalase activity in arsenic-exposed Drosophila melanogaster

Effect of Arsenic and Aframomum melegueta on Lipid Peroxidation (MDA) in Drosophila melanogaster

ANOVA results indicated a highly significant effect of treatments on MDA levels ($p = 0.0002$). Tukey's test revealed that arsenic exposure caused a marked increase in MDA compared with the control ($p = 0.0038$), reflecting lipid membrane damage due to excessive ROS generation (Fig. 8). Conversely, *Aframomum melegueta* administration significantly reduced MDA relative to the arsenic group ($p < 0.0001$), while the combined treatment also showed lower MDA compared with arsenic exposure ($p = 0.0010$). The difference between control and combined treatment was modest but significant ($p = 0.0010$). Collectively, these results demonstrate that *Aframomum melegueta* attenuated arsenic-induced lipid peroxidation, highlighting its antioxidative efficacy.

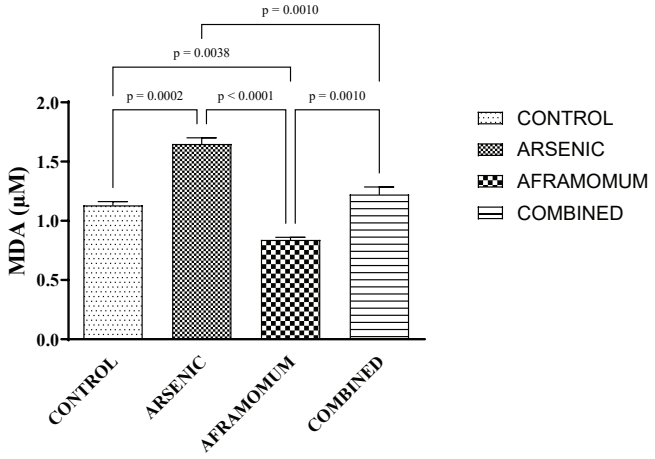

Figure 8: Effect of Aframomum melegueta extract on lipid peroxidation (MDA levels) in Drosophila melanogaster subjected to arsenic toxicity

Effect of Arsenic and Aframomum melegueta on Glutathione S-Transferase (GST) Activity in Drosophila melanogaster

ANOVA showed no significant differences across most treatment comparisons except at specific pairings. Tukey's test indicated no significant change between control and arsenic ($p = 0.8813$), or between control and *Aframomum* ($p = 0.7260$). However, the combined treatment exhibited significantly higher GST activity compared with arsenic exposure ($p = 0.0261$) and *Aframomum* alone ($p = 0.0168$). These data (Fig. 9) suggest that although individual treatments had limited impact, co-administration of *Aframomum melegueta* with arsenic enhanced detoxification enzyme activity, implying a synergistic upregulation of GST-mediated defense against oxidative insult.

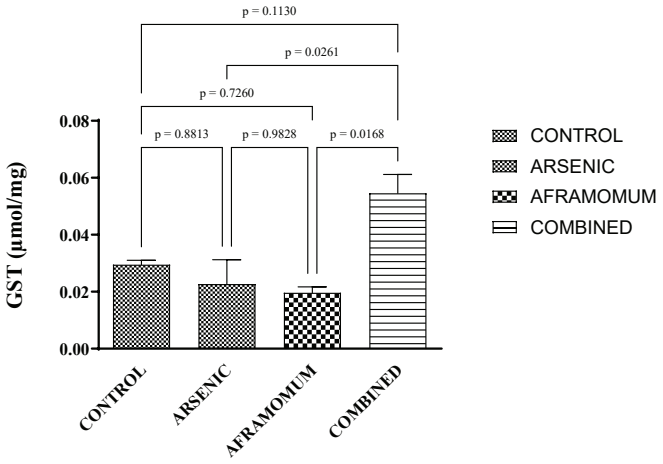

Figure 9: Effect of Aframomum melegueta extract on glutathione S-transferase (GST) activity in arsenic-treated Drosophila melanogaster

Effect of Arsenic and Aframomum melegueta on Total Thiol Levels in Drosophila melanogaster

Total thiol content showed treatment-dependent modulation. Arsenic-exposed flies exhibited decreased thiol levels compared with the control and combined treatment groups (Fig. 10), suggesting depletion of reduced thiols due to oxidative stress. *Aframomum melegueta* supplementation partially restored thiol levels, indicating antioxidant thiol preservation. Although exact p-values were not displayed, the distinct lettering denotes a statistically significant difference ($p < 0.05$) among treatments.

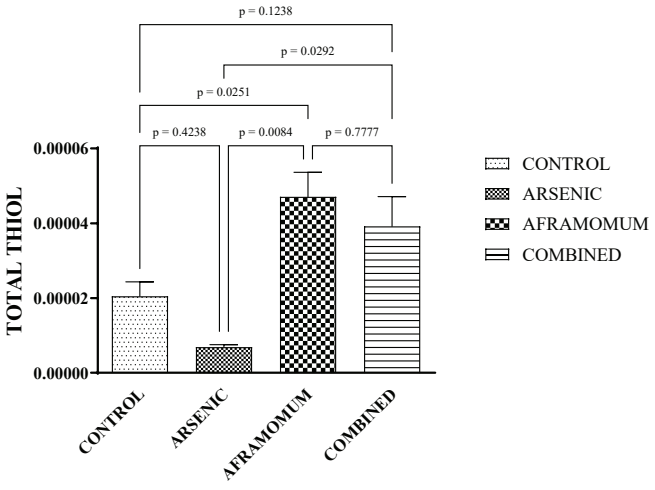

Figure 10: Effect of Aframomum melegueta extract on total thiol concentration in arsenic-exposed Drosophila melanogaster

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Effect of Arsenic and Aframomum melegueta on Total Protein Levels in Drosophila melanogaster

No significant treatment effect was observed in total protein concentration across groups. The ANOVA and Tukey's tests produced high p-values ($p = 0.9858$, $p = 0.2970$, $p = 0.6699$), showing that arsenic exposure and *Aframomum melegueta* treatment, either alone or combined, did not significantly alter overall protein content in *Drosophila melanogaster* (Fig. 11). This suggests that the interventions did not impair or stimulate general protein synthesis within the exposure period, although functional oxidative markers were altered.

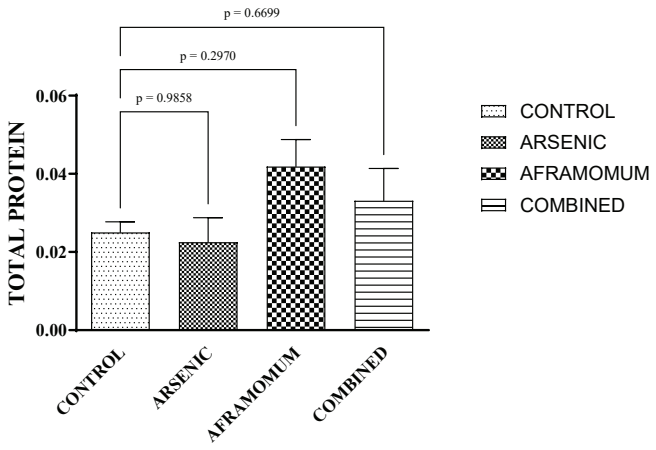

Figure 11: Effect of Aframomum melegueta extract on total protein concentration in Drosophila melanogaster under arsenic exposure

5. Discussion

The present study investigated the protective potential of *Aframomum melegueta* (grains of paradise) against arsenic-induced oxidative stress, biochemical alterations, and behavioral deficits in *Drosophila melanogaster*. The data revealed that arsenic exposure caused significant perturbations in key antioxidant and behavioral parameters [22], whereas *Aframomum melegueta* supplementation, either alone or in combination with arsenic, modulated these effects to varying degrees. Taken together, the findings highlight the phytochemical's ameliorative role against arsenic toxicity and emphasize its potential as a natural antioxidant and neuroprotective agent.

Arsenic exposure significantly suppressed catalase (CAT) activity [23] while markedly elevating lipid peroxidation levels (MDA) [24], confirming oxidative imbalance as a central mechanism of arsenic toxicity. Catalase, a primary hydrogen peroxide–scavenging enzyme, plays a vital role in cellular defense against reactive oxygen species (ROS) [25]. The significant decrease in catalase activity ($p = 0.0236$) in arsenic-exposed flies suggests enzyme inhibition via direct interaction of arsenic with sulfhydryl groups on catalase or through depletion of essential cofactors such as iron. Conversely, *Aframomum melegueta* treatment significantly restored catalase levels ($p = 0.0005$), both when administered alone and in combination with arsenic ($p = 0.0070$), demonstrating its capacity to reinforce enzymatic antioxidant defense. This observation aligns with previous reports that *Aframomum melegueta* extracts enhance catalase and superoxide dismutase (SOD) activities through their abundant phenolic and flavonoid content, including 6-gingerol, paradol, and shogaol analogs known to upregulate antioxidant gene expression and mitigate ROS accumulation [26,27].

Consistent with catalase suppression, arsenic exposure significantly increased MDA levels ($p = 0.0038$), reflecting enhanced lipid peroxidation and membrane damage [26]. Elevated MDA is a hallmark of oxidative injury resulting from peroxidation of polyunsaturated fatty acids [28]. The dramatic reduction in MDA upon *Aframomum melegueta* administration ($p < 0.0001$) indicates attenuation of lipid peroxidation, suggesting the Extract's potent ROS-scavenging activity. This is in agreement with studies by [29], who reported that *Aframomum melegueta* mitigates oxidative injury induced by heavy metals and xenobiotics in rodent tissues. The combined treatment's lowered MDA levels compared with arsenic alone ($p = 0.0010$) further supports a synergistic interaction between arsenic-induced oxidative triggers and *Aframomum*-mediated antioxidant response.

Glutathione S-transferase (GST), a critical phase II detoxification enzyme [30]the cytosolic glutathione S-transferase (GST, showed modest alterations across treatment groups. Although no significant difference was found between control and arsenic or *Aframomum* alone, the combined treatment group showed a significant elevation in GST activity compared with arsenic ($p = 0.0261$) and *Aframomum* alone ($p = 0.0168$). This suggests that co-exposure may have stimulated adaptive upregulation of GST-mediated conjugation pathways to facilitate detoxification of arsenic-derived electrophilic intermediates. The upsurge in GST could also reflect activation of the nuclear factor erythroid 2-related factor 2

(Nrf2) signaling pathway, which governs cellular redox homeostasis. The observed enhancement of GST activity may suggest involvement of redox-sensitive regulatory pathways such as Nrf2 [31]controlled production of oxidants in normal cells serves useful purposes to regulate signaling pathways. Reactive oxidants are counterbalanced by complex antioxidant defense systems regulated by a web of pathways to ensure that the response to oxidants is adequate for the body's needs. A recurrent theme in oxidant signaling and antioxidant defense is reactive cysteine thiol–based redox signaling. The nuclear factor erythroid 2–related factor 2 (Nrf2; however, this remains speculative, as direct molecular analyses were not performed in the present study.

Total thiol levels, an indirect measure of reduced glutathione (GSH) and protein thiols [32], were also modulated by treatment. Arsenic-exposed flies exhibited depleted thiol content, indicative of oxidative depletion and redox imbalance. *Aframomum melegueta* partially restored thiol concentration in the combined treatment group, consistent with its role in preserving intracellular thiol pools and reducing oxidative protein modification. These results affirm that *Aframomum melegueta* helps maintain cellular redox status by preventing excessive oxidation of cysteine residues and promoting glutathione regeneration [33].

Interestingly, total protein levels did not differ significantly across treatments ($p > 0.05$), implying that arsenic exposure and *Aframomum* intervention did not affect overall protein synthesis or degradation rates. The lack of change may reflect a selective oxidative impact restricted to specific enzymes and signaling proteins rather than a global suppression of protein metabolism.

Behavioral assays provided functional corroboration of biochemical findings. The survival rate assay revealed that arsenic exposure markedly reduced lifespan, while *Aframomum melegueta* alone or in combination modestly extended survival, particularly during later days of exposure. This partial restoration suggests that while the Extract mitigates chronic oxidative damage, it may not fully counteract arsenic's acute toxicity during early exposure phases. The observed survival improvement underscores *Aframomum's* role in delaying arsenic-induced senescence and improving overall vitality, consistent with its antioxidant action.

Arsenic exposure also caused notable impairments in neurobehavioral indices [34], including reduced climbing ability (negative geotaxis) and contraction parameters, reflecting motor dysfunction and neuromuscular weakness. The significant reduction in geotaxis performance ($p = 0.0168$) suggests neuronal toxicity in dopaminergic circuits, often implicated in motor control deficits in *Drosophila*. Although *Aframomum melegueta* did not completely restore geotaxis, the combined treatment showed partial improvement ($p = 0.0628–0.9729$), indicative of a neuroprotective trend. Similarly, contraction parameters were significantly influenced ($p = 0.0408$), where arsenic exposure impaired muscular movement amplitude, while *Aframomum* treatment showed mild recovery. These outcomes may be linked to the Extract's ability to preserve neuromuscular function through antioxidant modulation and possibly through the stabilization of acetylcholine levels, as suggested in related studies on plant-derived neuroprotectants [35]. Although oxidative stress is known to impair neuromuscular and cholinergic function [36], acetylcholinesterase activity and neurotransmitter dynamics were not evaluated in this study; therefore, the neurobehavioral improvements observed should be interpreted at a functional rather than mechanistic level.

Locomotor distance and speed further reflected arsenic-induced neurotoxicity. Arsenic significantly reduced locomotor distance, whereas *Aframomum melegueta* treatment restored movement capacity ($p = 0.0280$), indicating a functional recovery of neuromotor coordination. The partial, non-significant changes in locomotor speed ($p = 0.0400$ overall but $p > 0.44$ post hoc) suggest that while *Aframomum* improved endurance and activity span, it may not have markedly influenced velocity per se. Taken together, these behavioral findings reinforce the biochemical evidence that *Aframomum melegueta* exerts restorative effects on arsenic-induced oxidative and neuromuscular dysfunction.

Mechanistically, the ameliorative effects of *Aframomum melegueta* may be attributed to its rich repertoire of phenolic constituents and essential oils, such as 6-paradol, 6-shogaol, and 6-gingerol. These compounds are known to scavenge ROS, enhance endogenous antioxidant enzyme expression, and stabilize cellular membranes. Furthermore, the modulation of detoxification enzymes like GST and restoration of thiol levels point toward activation of the Nrf2–

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ARE signaling cascade, a key regulator of cellular defense. The protective trends observed across multiple parameters suggest that *Aframomum melegueta* acts both as a direct antioxidant and as a modulator of redox-sensitive signaling networks.

This study establishes that arsenic induces oxidative and behavioral toxicity in *Drosophila melanogaster*, characterized by elevated lipid peroxidation, suppression of antioxidant enzymes, and impaired locomotor performance. *Aframomum melegueta* effectively mitigated these effects, restoring catalase and thiol activity, reducing lipid peroxidation, enhancing GST function, and improving survival and motor performance. The findings collectively affirm the antioxidative and neuroprotective potential of *Aframomum melegueta* against heavy metal–induced toxicity. These results support its potential utility as a dietary or therapeutic agent for mitigating oxidative stress-related disorders. Further molecular studies focusing on gene expression of antioxidant enzymes, Nrf2 pathway activation, and dose–response profiling in higher models would provide deeper insights into its mechanistic efficacy and translational relevance.

Behavioral outcomes demonstrated greater variability than biochemical endpoints, which is characteristic of *Drosophila* locomotor assays. While arsenic exposure consistently impaired motor performance, the modulatory effects of Aframomum melegueta were modest and, in some assays, did not reach statistical significance. These findings suggest partial functional protection rather than complete neurobehavioral restoration.

The inclusion of HPLC-based phytochemical profiling strengthens the interpretation of the biological effects observed in this study. The chromatographic fingerprint confirms that the ethanol extract of *Aframomum melegueta* contains multiple bioactive constituents, likely including phenolic and gingerol-related compounds previously associated with antioxidant and neuroprotective activities. These compounds may collectively contribute to the attenuation of lipid peroxidation, restoration of antioxidant enzymes, and partial improvement in neurobehavioral outcomes observed following arsenic exposure. However, while HPLC profiling confirms extract complexity and reproducibility, definitive compound identification and quantification would require further targeted analyses using reference standards or mass spectrometry.

6. Study Limitations

A key limitation of this study is that the molecular mechanisms underlying the observed biochemical and behavioral effects were not examined, particularly gene expression profiles of antioxidant enzymes and the involvement of the Nrf2 signaling pathway. Nevertheless, these findings provide preliminary in vivo evidence that supports further targeted mechanistic investigations. Although HPLC profiling was performed to characterize the Extract, individual phytochemical constituents were not fully identified or quantified against reference standards. Therefore, extract standardization remains partial, and future studies should incorporate quantitative phytochemical analysis to improve translational and nutraceutical applicability. Future studies should therefore investigate these mechanistic pathways, explore dose–response relationships, and extend the findings to mammalian models to validate the translational potential of *Aframomum melegueta* as a therapeutic candidate against heavy metal–induced oxidative and neurobehavioral toxicity.

7. Conclusion

This study demonstrated that arsenic exposure induces marked oxidative and neurobehavioral disturbances in *Drosophila melanogaster*, characterized by reduced catalase activity, elevated lipid peroxidation, depletion of thiol reserves, and impaired locomotor performance. These findings establish *Aframomum melegueta* as a modulator of arsenic-induced oxidative and neurobehavioral toxicity in *Drosophila melanogaster*, providing a foundation for future mechanistic and translational studies by enhancing antioxidant enzyme activities, restoring thiol balance, and reducing lipid peroxidation, thereby protecting cellular integrity and improving behavioral outcomes. Although total protein levels remained unchanged, the modulation of catalase, GST, and thiol parameters underscores the Extract's potent redox-stabilizing capacity. Behavioral assays further confirmed partial recovery of motor coordination and survival, indicating that *Aframomum melegueta* confers both biochemical and functional protection against arsenic toxicity. These

findings highlight the therapeutic promise of *Aframomum melegueta* as a natural antioxidant and neuroprotective agent. Future studies should investigate its molecular targets, optimal dosing, and long-term safety to facilitate translational applications in metal-induced oxidative stress and neurodegenerative conditions.

Declarations

Ethical Statement:

Experiments involving *Drosophila melanogaster* are exempt from institutional ethical approval, as the species is an invertebrate model not covered under regulations governing vertebrate animal research. All experimental procedures were, however, conducted in accordance with internationally accepted standards for the humane use of invertebrate organisms in scientific research.

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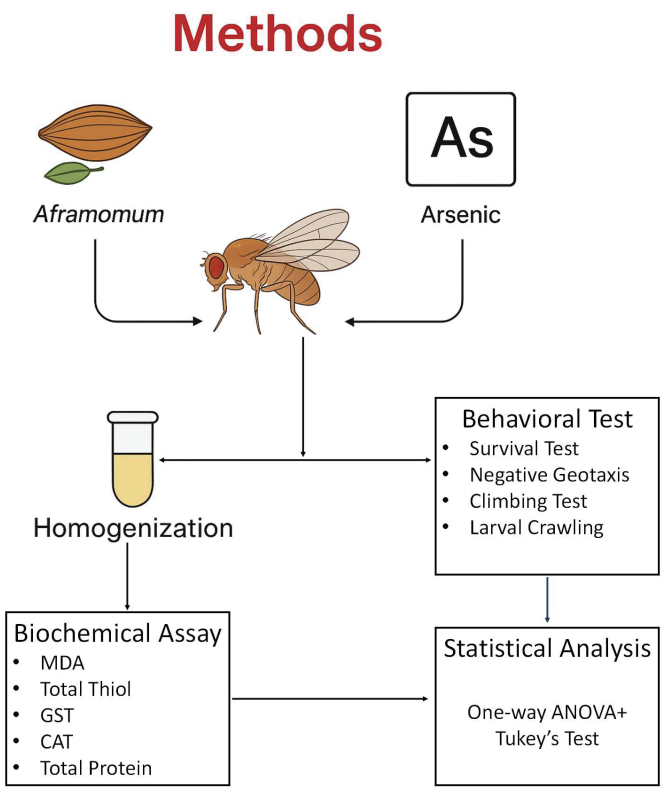
Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphical abstract



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