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Original Article

Evaluating the effects of sodium metabisulfite on the cognitive and motor function in *Drosophila melanogaster*

John SB. Nas[1,2] and Paul MB. Medina[1*]

[1]Biological Models Laboratory, Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, Philippines; [2]Department of Biology, College of Arts and Sciences, University of the Philippines Manila, Manila, Philippines

*Corresponding author: pmbmedina@post.upm.edu.ph

Abstract

Sodium metabisulfite is widely used as a preservative in many food and beverage products, yet its potential effects on cognitive and motor functions at low concentrations remain poorly understood. Evaluating learning, short-term memory, and motor activity is essential, as these functions are critical indicators of neurological health and could be impacted by low-level exposure to sodium metabisulfite. The aim of this study was to investigate the effects of sublethal concentrations of sodium metabisulfite on cognitive and motor functions using *Drosophila melanogaster* (fruit flies) as the model organism. Different levels of sodium metabisulfite were administered to male and female fruit flies, and their learning and short-term memory were observed. Additionally, their climbing activity with and without stressors (heat shock, ultraviolet A exposure, or energy deprivation) was examined. Our findings indicated that sodium metabisulfite did not impair learning, short-term memory, or motor activity. Furthermore, sodium metabisulfite did not affect the motor activity of fruit flies under heat, ultraviolet A, and energy-deprived conditions. In conclusion, our results suggested that the sublethal concentration of sodium metabisulfite did not harm cognitive and motor functions and did not exacerbate the effects of environmental stressors.

Keywords: Sodium metabisulfite, *Drosophila melanogaster*, cognition, motor function, stress

Introduction

Sodium metabisulfite is a widely utilized reducing agent in both industrial and scientific settings. Its molecular structure consists of two sodium ions and one bisulfite ion. Upon reaction with water, sodium metabisulfite forms sulfite (SO_3^{2-}), which mediates its primary biological effects [1]. Its reduction potential of -0.66 V makes it an effective reducing agent for metals, halogens, and organic compounds [1]. The presence of sulfur dioxide (SO_2) in its molecular structure is crucial for its reducing capabilities, as SO_2 has a high electron affinity and can readily donate electrons to other molecules, facilitating their reduction. The solubility of SO_2 in water increases with temperature, and its reactivity is pH-dependent, enhancing its utility in various applications [1]. Moreover, the rapid metabolic clearance of sulfites has been demonstrated in different animal models [2]. Following oral administration in dogs and rats, as well as intravenous (IV) treatment in rabbits, rats, and rhesus monkeys, sulfites are swiftly processed [2,3]. While the liver metabolizes a portion of the sulfite, a small amount may enter the bloodstream and approximately 10% or less of the orally administered sulfite is excreted unaltered in the urine [2]. Sulfite oxidase converts sulfite to sulfate upon ingestion, inhalation, or injection. A study showed that sulfite

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oxidase-deficient rats administered sulfite exhibited elevated copper levels in the kidney, while iron and zinc levels in the liver and kidney were comparable to both untreated sulfite oxidase-deficient rats and normal rats [4].

One of the principal applications of sodium metabisulfite is as a preservative and antioxidant in the food industry, where it inhibits bacterial growth and prevents oxidative degradation of food and beverages [5]. It is also employed in the production of industrial products such as paper, textiles, and leather, as well as in the synthesis of organic compounds, including dyes, pharmaceuticals, and plastics [6]. Additionally, its ability to reduce metal ions to their elemental form makes it useful in metal extraction from ores [6]. Bisulfite engages in significant reactions with biomolecules, such as sulfonation and autooxidation, leading to the generation of free radicals, and addition to cytosine [7]. Sulfonation products are long-lived in vivo and may exhibit high reactivity, while autooxidation products can initiate lipid peroxidation, thereby damaging plasma membranes [7]. Furthermore, bisulfite can react with nucleic acids, converting cytosine to uracil, resulting in mutational events [8]. As a nucleophile, sulfite can also donate electrons to carbonyl compounds, leading to structural alterations in carbohydrates such as aldose and ketose [8].

Concerns have been raised regarding the potential biological effects of sulfite. Sulfite can interact with proteins, lipids, and DNA, which may result in various health impacts [9]. High doses of sulfite have been linked to asthma, allergic reactions, headaches, and seizures [10,11]. It has been suggested that sulfite consumption is linked with inflammation and cancer development [12]. However, low levels of serum bisulfite play a beneficial role in humans. These include protecting cells from damage, lowering blood pressure, reducing inflammation, relaxing blood vessels, inhibiting the contraction of smooth muscle cells in blood vessels, maintaining a healthy cardiovascular structure, and ensuring proper function of channels in the heart [13]. Hence, regulatory agencies worldwide oversee its use in food and other products to ensure safety.

Many food and beverage products contain artificial preservatives like sodium metabisulfite, which is commonly used to prolong shelf life and prevent spoilage [5-8]. Although the detrimental health effects of high doses of sodium metabisulfite have been documented in animals, the impact of consuming sublethal or low concentrations remains poorly understood, particularly in relation to cognitive and motor functions. Understanding these effects is crucial for evaluating the safety of long-term, low-level exposure to this preservative in humans. To explore this, we used *Drosophila melanogaster* (fruit flies) as the model organism due to their well-established role in genetic, neurological, and behavioral studies [14]. Fruit flies share many genetic and physiological similarities with humans, and their relatively short lifespan allows for the rapid observation of generational effects [14,15]. Additionally, their simple nervous system makes them an ideal model for studying the basic mechanisms underlying cognitive and motor functions. The aim of this study was to investigate the effects of sublethal concentrations of sodium metabisulfite on cognitive and motor functions in *D. melanogaster*. The stress tolerance under various environmental stressors (heat shock, ultraviolet A (UVA) exposure, and energy deprivation) was also examined to determine whether low doses of sodium metabisulfite could exacerbate stress responses.

Methods

Maintenance and husbandry of *Drosophila*

Wild-type Oregon-R *D. melanogaster* was cultured in vials containing Formula 4-24 Instant *Drosophila* Medium (Carolina Biological Supply, North Carolina, USA) at a constant temperature of 25°C. To prepare the growth medium, equal volumes of Formula 424 and water were added to each vial. The vials were then allowed to sit for a short period of time to allow the medium to solidify. Finally, each vial was inoculated with approximately six grains of yeast. To maintain the wild-type Oregon-R strain of *D. melanogaster*, flies were transferred to fresh culture vials every four days to prevent the crossbreeding of F1 offspring [14]. To ensure that the flies were of the same age, male and female flies 0–24 hours post-eclosion were used in the subsequent assays.

Storage and preparation of chemicals

Sodium metabisulfite (Kemrad Incorporated, Quezon City, Philippines) was stored at ambient temperature. In preparing the sodium metabisulfite solution, the compound was dissolved in

water following the final volume of their desired concentration. The prepared solution was stored in the fridge at a temperature of 4°C before use. Freshly prepared solutions of varying concentrations were used for the succeeding experiments.

Sublethal assay

Thirty male and female *D. melanogaster* were administered varying doses (from 2.5 to 20 mg/L) of sodium metabisulfite incorporated into their diet. The daily survival rate of the fruit flies was recorded until five days post-eclosion to assess the chronic lethality of sodium metabisulfite. A survival rate exceeding 90% was classified as indicative of sublethal concentrations [14]. The maximum sublethal concentration identified in this assay was utilized as the highest concentration in subsequent experiments, with two-fold and four-fold lower concentrations designated as the medium and low concentrations, respectively.

Aversive phototaxis suppression assay—learning assay

After five days of exposure to sodium metabisulfite, the flies were subjected to an aversive phototaxis suppression assay as previously described [15]. This assay used a T-maze to assess the phototactic behavior and associative learning of the flies under aversive conditions [15].

Individual flies were introduced into the darkened chamber of the T-maze. The flies were acclimatized for 30 seconds, after which the opposing chamber was opened, exposing the pin light. Flies exhibiting positive phototaxis entered the lighted chamber within 45 seconds. These flies were retained for subsequent training, while flies demonstrating negative phototaxis were excluded from further experimentation.

Positively phototactic flies were returned to the darkened chamber for an additional 30-second acclimation period. A filter paper treated with quinine solution was then placed inside the lighted chamber as an aversive stimulus. The central partition was reopened, and the fly's behavior was observed for another 45 seconds. This conditioning was repeated 15 times. Flies avoiding the lighted chamber were recorded as "pass", indicating successful aversive learning. The percentage of successful trials within a consecutive five-trial block was defined as PC0 (0 hours post-conditioning).

Subsequently, flies were returned to their housing for a six-hour interval before undergoing identical testing to assess short-term memory. The corresponding percentage of successful trials was designated as PC6 (six hours post-conditioning). The entire procedure was replicated independently with three distinct cohorts of flies.

Negative geotaxis assay

Fruit flies exposed to varying concentrations of sodium metabisulfite underwent negative geotaxis assays, as outlined previously [16]. Experimental groups were independently housed in sealed vials. In each vial, flies were dislodged to its base through gentle tapping. Subsequent climbing behavior was monitored for one minute, with the number of flies surpassing the 8 cm mark recorded at 10-second intervals. Climbing performance was expressed as a percentage of non-motor-impaired flies. Individuals exhibiting reduced mobility or a propensity to remain at the vial base were classified as motor impaired. To enhance assay robustness, each experimental group was subjected to three replicate trials.

Stress assays

Heat stress

To investigate the impact of sodium metabisulfite on motor function under heat stress, 30 male and 30 female *D. melanogaster* were exposed to a range of sodium metabisulfite concentrations (0, 0.625, 1.25, and 2.5 mg/L) daily. Concurrently, flies were subjected to a 60-minute heat stressor at 37°C each day [17]. Motor function was assessed via negative geotaxis assay, quantifying climbing performance as previously detailed.

UVA stress

The motor function of *D. melanogaster* under UVA stress was evaluated by exposing 30 male and 30 female flies to varying concentrations of sodium metabisulfite (0, 0.625, 1.25, and 2.5 mg/L) daily. Simultaneously, flies were irradiated with 360 nm UVA light for 60 mins daily

[18]. The influence of sodium metabisulfite on motor function was determined by measuring climbing performance using the negative geotaxis assay.

Energy deprivation assay (acute sodium metabisulfite supplementation)

Cohorts of 30 male and 30 female flies were fed diets containing varying concentrations of sodium metabisulfite (0, 0.625, 1.25, and 2.5 mg/L) for five days. Subsequently, both diet and sodium metabisulfite were removed, and flies were provided with distilled water until mortality. The motor function was evaluated during the starved state using the negative geotaxis assay to quantify their climbing activity [14].

Energy deprivation assay (chronic sodium metabisulfite supplementation)

Similar to the acute supplementation group, 30 male and 30 female flies were fed diets containing varying concentrations of sodium metabisulfite (0, 0.625, 1.25, and 2.5 mg/L) for five days. However, following this period, only the diet was removed, while sodium metabisulfite exposure continued until the death of all flies. The motor function was assessed throughout the energy deprivation period using the negative geotaxis assay by quantifying their climbing performance [14].

Statistical analysis

The data for learning index, short-term memory index, and climbing pass rate were calculated as the average of three trials and presented as mean ± standard deviation (SD). GraphPad Prism version 9 (GraphPad Software, San Diego, USA) was used for statistical analysis, with significance set at p=0.05. For parametric data, analysis of variance (ANOVA) was used, accompanied by Tukey's test for post-hoc analysis. For the non-parametric data, the Kruskal-Wallis test was performed, followed by Dunn's test for post-hoc analysis.

Results

Sublethal concentration of sodium metabisulfite in *D. melanogaster*

In this experiment, the sublethal concentration of sodium metabisulfite for male flies was found to be 2.5 mg/L. This concentration showed a 100% survival rate over a five-day exposure period, which was comparable to the untreated control group (**Figure 1A**). Conversely, exposure to higher concentrations of sodium metabisulfite (5, 10, and 20 mg/L) resulted in survival rates dropping below 90%, suggesting the potential for chronic toxicity at these elevated levels.

In female flies, the sublethal concentration was determined to be 2.5 mg/L (**Figure 1B**). Exposure to higher concentrations of sodium metabisulfite resulted in survival rates decreasing below 90% after five days, indicating a similar threshold for chronic toxicity as observed in male counterparts.

Effects of sodium metabisulfite on the cognitive function of *D. melanogaster*

About 33% of phototactic male flies demonstrated the ability to learn light aversion due to quinine exposure. The learning performance of male flies administered various concentrations of sodium metabisulfite was comparable to that of untreated male flies (**Figure 2A**). However, their aversion to light decreased to 29% after six hours. This reduction in the short-term memory index in male flies after six hours was also comparable to the male flies treated with sodium metabisulfite (**Figure 2B**).

While 36% of the phototactic female flies have successfully learned light aversion, the learning performance of the female flies fed with sodium metabisulfite did not vary significantly, regardless of whether they were fed varying concentrations or not (**Figure 2C**). Nevertheless, the aversion response in female flies declined to 19% after six hours. This decline in aversion among treated female flies was comparable to those given sodium metabisulfite (**Figure 2D**). When both sexes were combined, 34.8% of the flies exhibited learned aversion. The number of flies displaying this behavior immediately after training (0 hours) and six hours post-training were comparable to those treated with varying concentrations of sodium metabisulfite (**Figure 2G** and **2H**). A similar pattern was observed in the short-term memory assessment for both sexes (**Figure 2I**).

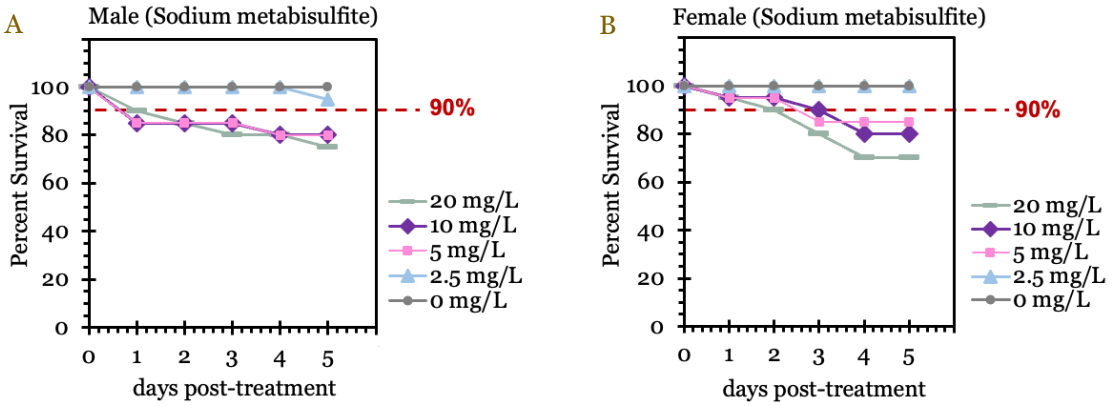

Figure 1. Sublethal concentration of sodium metabisulfite in *D. melanogaster*. Thirty male (A) and thirty female (B) flies were given varying concentrations of sodium metabisulfite (0, 2.5, 5, 10, and 20 mg/L) in their diet for five days. The survival rate was recorded daily. The sublethal concentration was determined as the highest amount of sodium metabisulfite with at least 90% survival after five days of treatment.

Effects of sodium metabisulfite on the motor function of *D. melanogaster* without stress

The locomotor behavior of male and female flies was assessed under the influence of varying sodium metabisulfite concentrations and compared to untreated controls with the results presented in **Figure 3**. Climbing activity, a measure of locomotor function, remained consistent across all sodium metabisulfite treatment groups for both sexes when compared to their respective controls (**Figures 3A** and **3C**). These indicated that the presence of sodium metabisulfite, within the tested concentration range, did not significantly impact the flies' ability to climb.

It is important to note that a general decline in climbing performance was observed over time for both treated and untreated flies (**Figures 3B** and **3D**). This age-related or experimental condition-related decrease in motor activity was not exacerbated by sodium metabisulfite exposure. Therefore, the observed reduction in climbing pass rate cannot be attributed to the effects of sodium metabisulfite. Both sexes exhibited a comparable decline in motor activity after 30 days, regardless of whether they were fed sodium metabisulfite (**Figure 3E**). The proportion of flies able to climb the 8 cm mark was similar across the different concentrations of sodium metabisulfite (**Figure 3F**).

Effects of sodium metabisulfite on the motor function of *D. melanogaster* under heat stress

The effects of sodium metabisulfite on the motor function of *D. melanogaster* under heat stress are presented in **Figure 4**. The climbing activity of male and female flies was significantly reduced under heat stress, with a 21.9% and 27.0% decrease observed for males and females, respectively (**Figures 4A** and **4D**). However, male flies administered with various concentrations of sodium metabisulfite showed climbing activity comparable to that of untreated control flies (**Figure 4B**). The general decline in climbing pass rate over time was consistent across all groups (**Figure 4C**), indicating that sodium metabisulfite did not specifically impair the motor activity of male flies under heat stress conditions.

Female flies also showed a similar pattern, with climbing activity comparable to that of untreated counterparts, even under heat stress (**Figures 4E** and **4F**). The observed reduction in climbing pass rate over time was not influenced by sodium metabisulfite exposure, suggesting that the compound does not negatively impact motor activity in female flies under these stressful conditions.

The combined climbing activity of both sexes was 24.5% lower under heat stress ($p<0.05$), as presented in **Figure 4G**. Furthermore, the combined climbing activity 18 days after sodium metabisulfite treatment remained similar to that of the untreated group (**Figures 4H** and **4I**).

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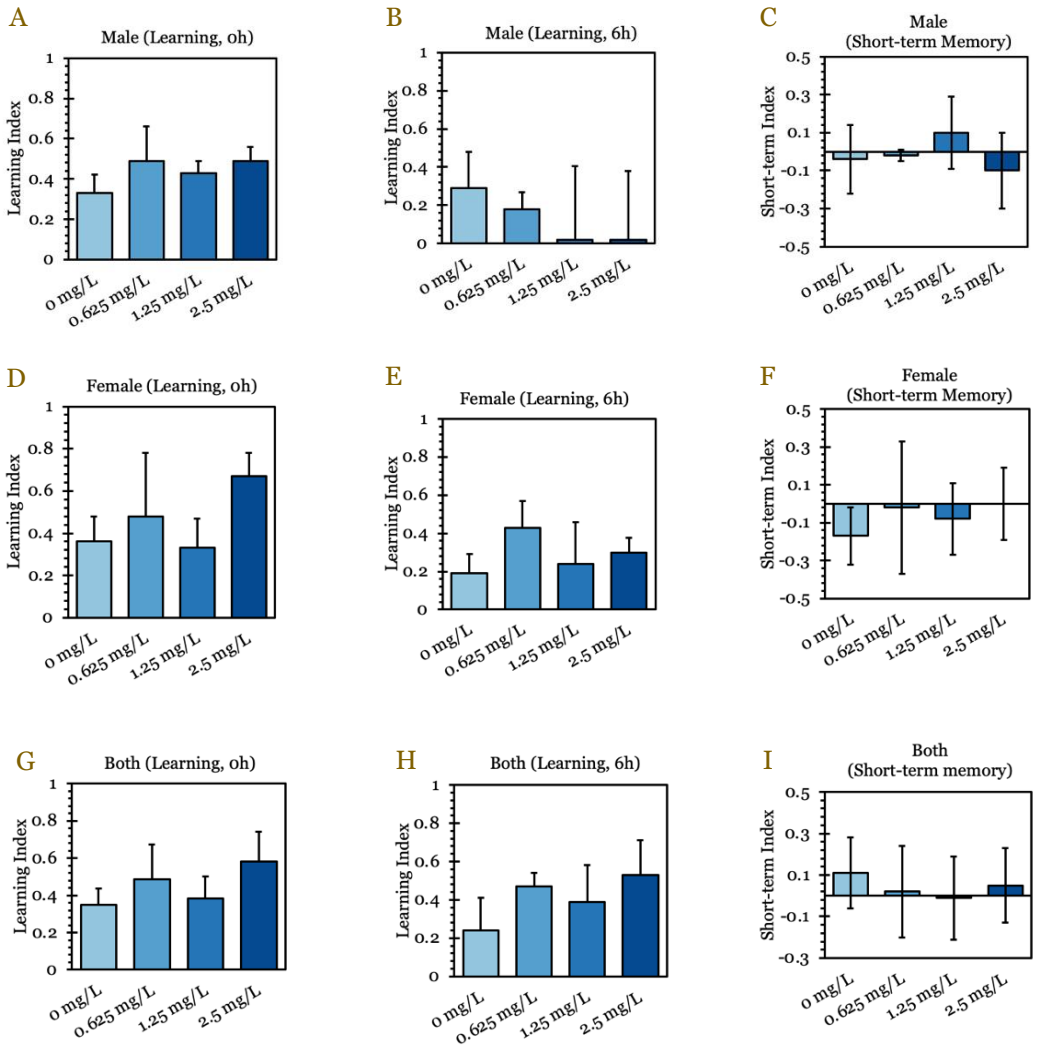

Figure 2. Sodium metabisulfite does not affect learning and short-term memory in *D. melanogaster*. Thirty male and thirty female flies were pre-treated with varying concentrations of (0, 0.625, 1.25, and 2.5 mg/L) sodium metabisulfite for five days. The learning after 0 h (A), learning after 6 h (B), and short-term memory of male flies (C) were determined using an aversive phototaxis suppression assay. The learning after 0 h (D), learning after 6 h (E), and short-term memory of female flies (F) were determined using the same assay. The combined sexes (male and female flies) were also assessed for learning after 0 h (G), learning after 6 h (H), and short-term memory (I).

Effects of sodium metabisulfite on the motor function of *D. melanogaster* under UVA stress

The effects of sodium metabisulfite on the motor function of *D. melanogaster* under UVA stress are presented in **Figure 5**. UVA stress significantly reduced the climbing activity of both male and female flies, with a 30.8% decrease for males and a 31.7% decrease for females (**Figure 5A** and **5D**). However, male flies administered with various concentrations of sodium metabisulfite showed climbing activity comparable to that of untreated control flies (**Figure 5B**). The general decline in climbing pass rate over time was consistent across all groups (**Figure 5C**), indicating that sodium metabisulfite did not specifically impair the motor activity of male flies under UVA stress conditions.

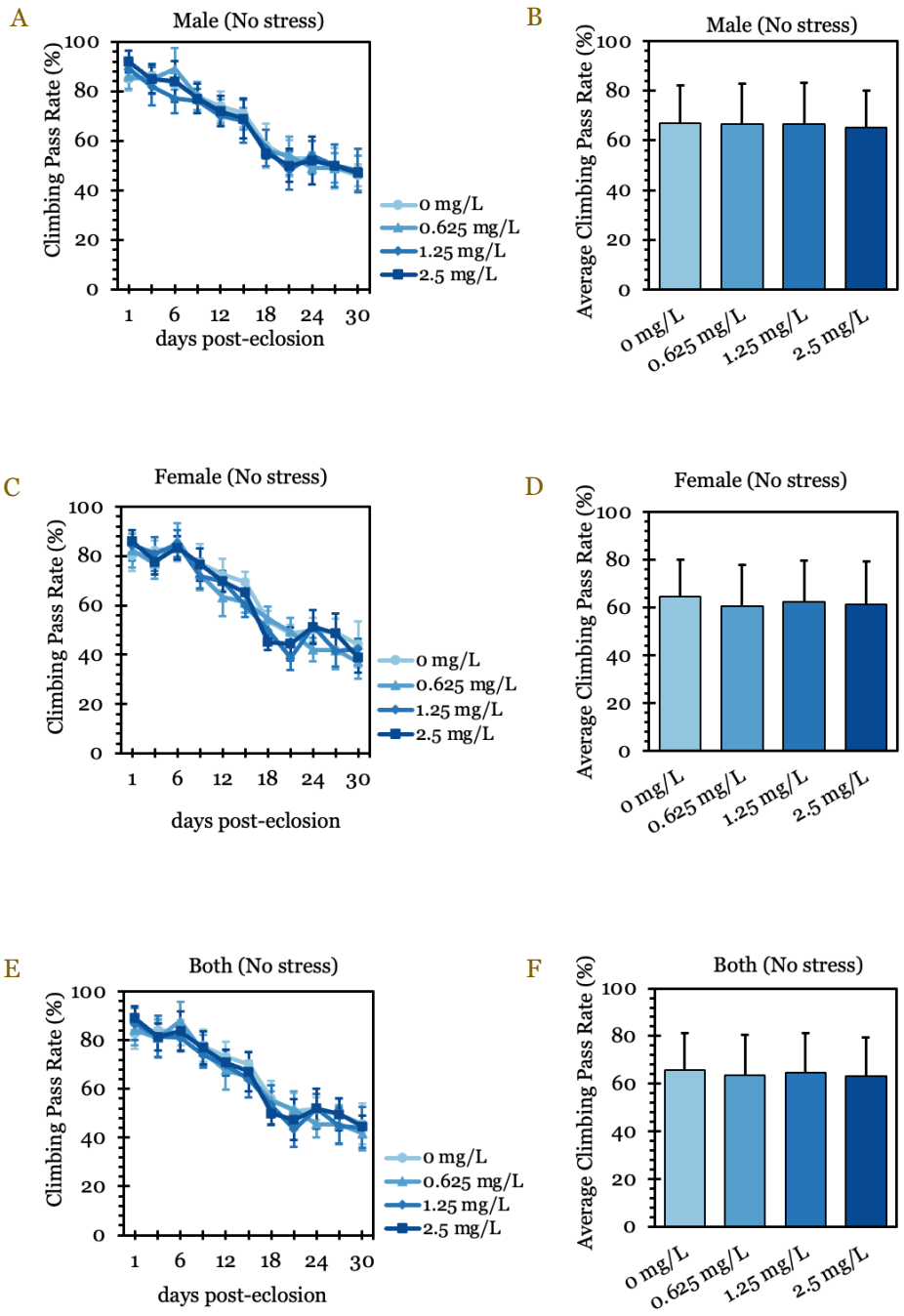

Figure 3. Sodium metabisulfite does not affect the climbing activity of *D. melanogaster*. Thirty male and thirty female flies were fed with varying concentrations of (0, 0.625, 1.25, and 2.5 mg/L) sodium metabisulfite. The daily climbing activity (A) and average climbing activity of male flies (B) were determined using a negative geotaxis assay. The daily climbing activity (C) and average climbing activity of female flies (D) were evaluated using the same assay. The combined sexes were also assessed for daily climbing activity (E) and average climbing activity (F).

Female flies also showed a similar pattern, with climbing activity comparable to that of untreated counterparts, even under UVA stress (**Figure 5E** and **5F**). The observed reduction in climbing pass rate over time was not influenced by sodium metabisulfite exposure, suggesting that the compound does not negatively impact motor activity in female flies under these stressful conditions.

When looking at both sexes together, there was a 31.3% drop in climbing activity under UVA stress ($p<0.05$), as presented in **Figure 5G**. The trend and average climbing activity of flies fed

sodium metabisulfite under UVA stress didn't significantly differ from the untreated group (**Figures 5H** and **5I**).

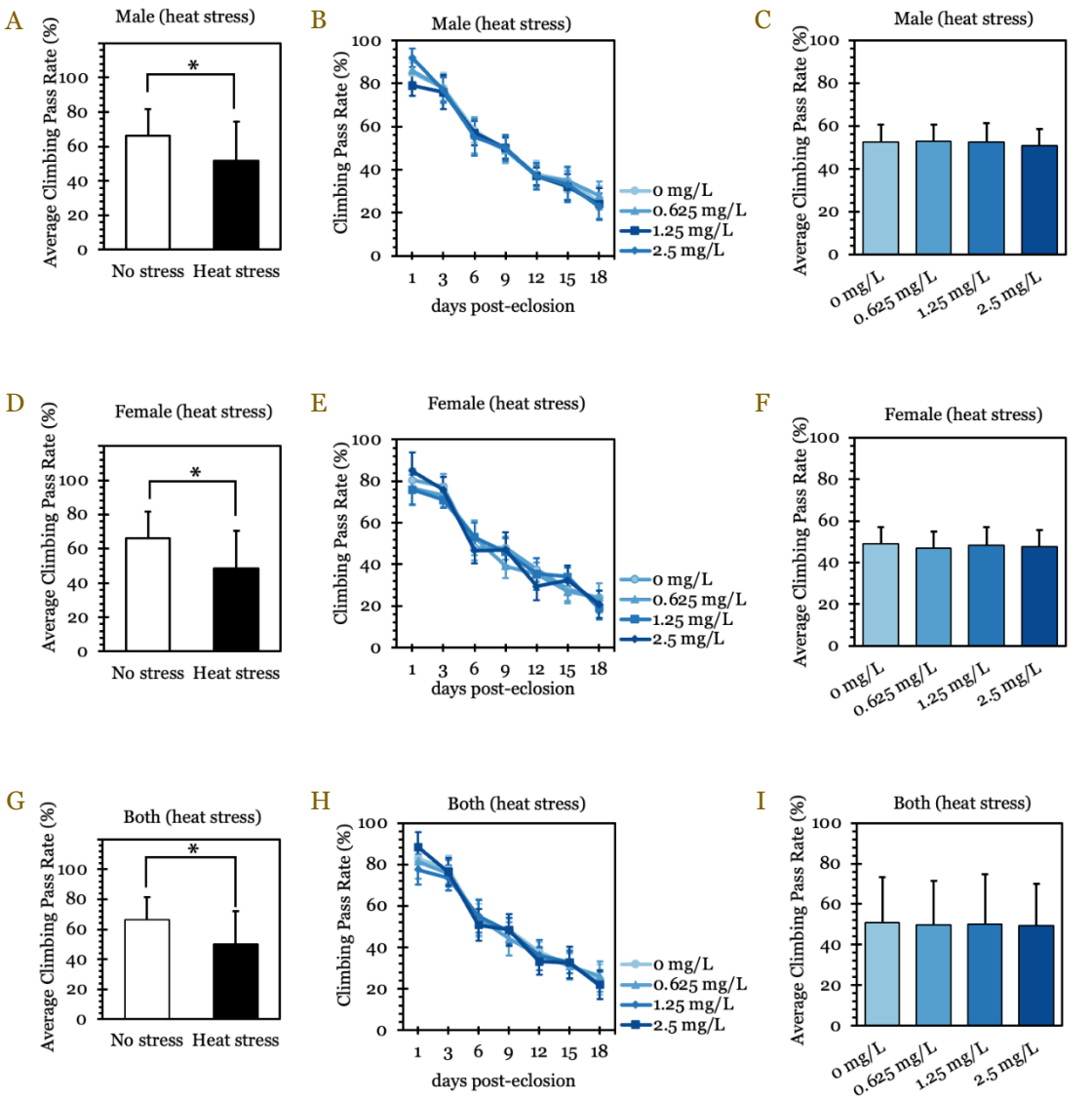

Figure 4. Sodium metabisulfite does not affect the climbing activity of *D. melanogaster* under heat stress. Thirty male and thirty female flies were fed with varying concentrations of (0, 0.625, 1.25, and 2.5 mg/L) sodium metabisulfite every day while they were exposed to a high temperature, 37°C for 60 minutes daily, to induce heat stress. The effect of heat stress on the climbing activity of the untreated male flies (A), daily climbing activity (B), and average climbing activity of male flies (C) were determined using negative geotaxis assay while the flies were under heat stress. The effect of heat stress on the climbing activity of the untreated female flies (D), daily climbing activity (E), and average climbing activity of female flies under heat stress (F) were evaluated using the same assay. The combined sexes (male and female flies) were also assessed for effects of heat stress on climbing activity (G), daily climbing activity (H), and average climbing activity of both male and female flies (I). *Mean±SD has a significant difference at $p<0.05$.

Effects of sodium metabisulfite consumption on the motor function of *D. melanogaster* under energy deprivation

The effects of sodium metabisulfite consumption on the motor function of *D. melanogaster* under energy deprivation are presented in **Figure 6** and **Figure 7**. Energy deprivation resulted in a significant decline in locomotor function for both male and female flies, as evidenced by a substantial 36.5% and 31.7% decrease in climbing activity, respectively (**Figures 6A**, **6D**, **7A**, and **7D**). This reduction in climbing ability was indicative of a broader impairment in motor function, likely due to the physiological stress associated with energy depletion.

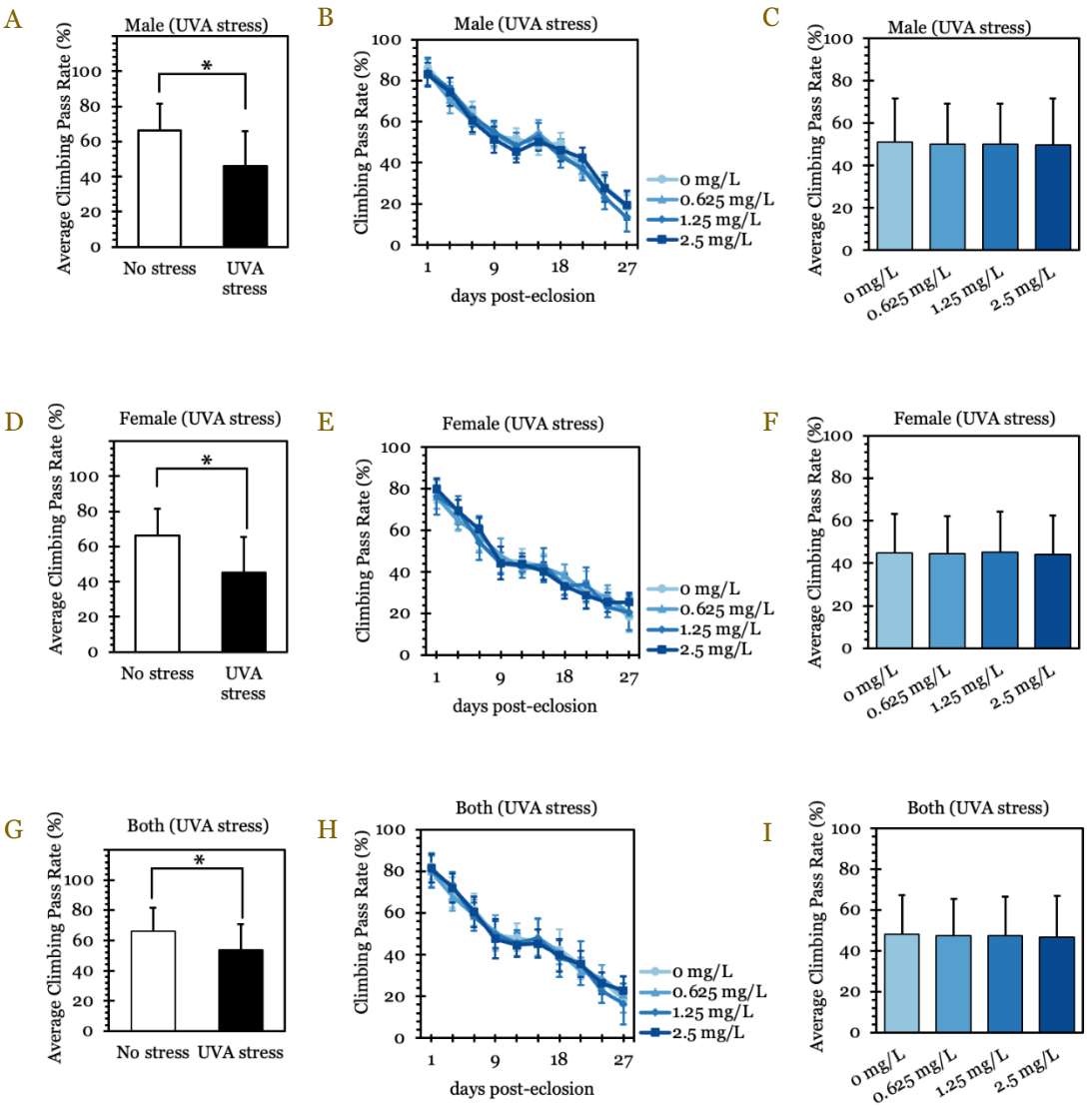

Figure 5. Sodium metabisulfite does not affect the climbing activity of *D. melanogaster* under UVA stress. Thirty male and thirty female flies were fed with varying concentrations of (0, 0.625, 1.25, and 2.5 mg/L) sodium metabisulfite every day while they were irradiated with 360nm UV for 60 minutes daily to induce UVA stress. The effect of UVA stress on the climbing activity of the untreated male flies (A), daily climbing activity (B), and average climbing activity of male flies (C) were determined using negative geotaxis assay while the flies were under UVA stress. The effect of UVA stress on the climbing activity of the untreated female flies (D), daily climbing activity (E), and average climbing activity of female flies under UVA stress (F) were evaluated using the same assay. The effects of UVA stress on climbing activity (G), daily climbing activity (H), and average climbing activity of both male and female flies (I) were also assessed. *Mean±SD has a significant difference at $p<0.05$.

Preconditioning the flies with sodium metabisulfite for five days prior to energy deprivation did not affect the locomotor function in male flies. These males exhibited climbing activity comparable to untreated control flies subjected to the same energy deprivation conditions (**Figure 6B** and **6C**). The general decline in climbing pass rate over time was consistent across all groups, indicating that sodium metabisulfite did not specifically impair the motor activity of male flies under energy deprivation conditions.

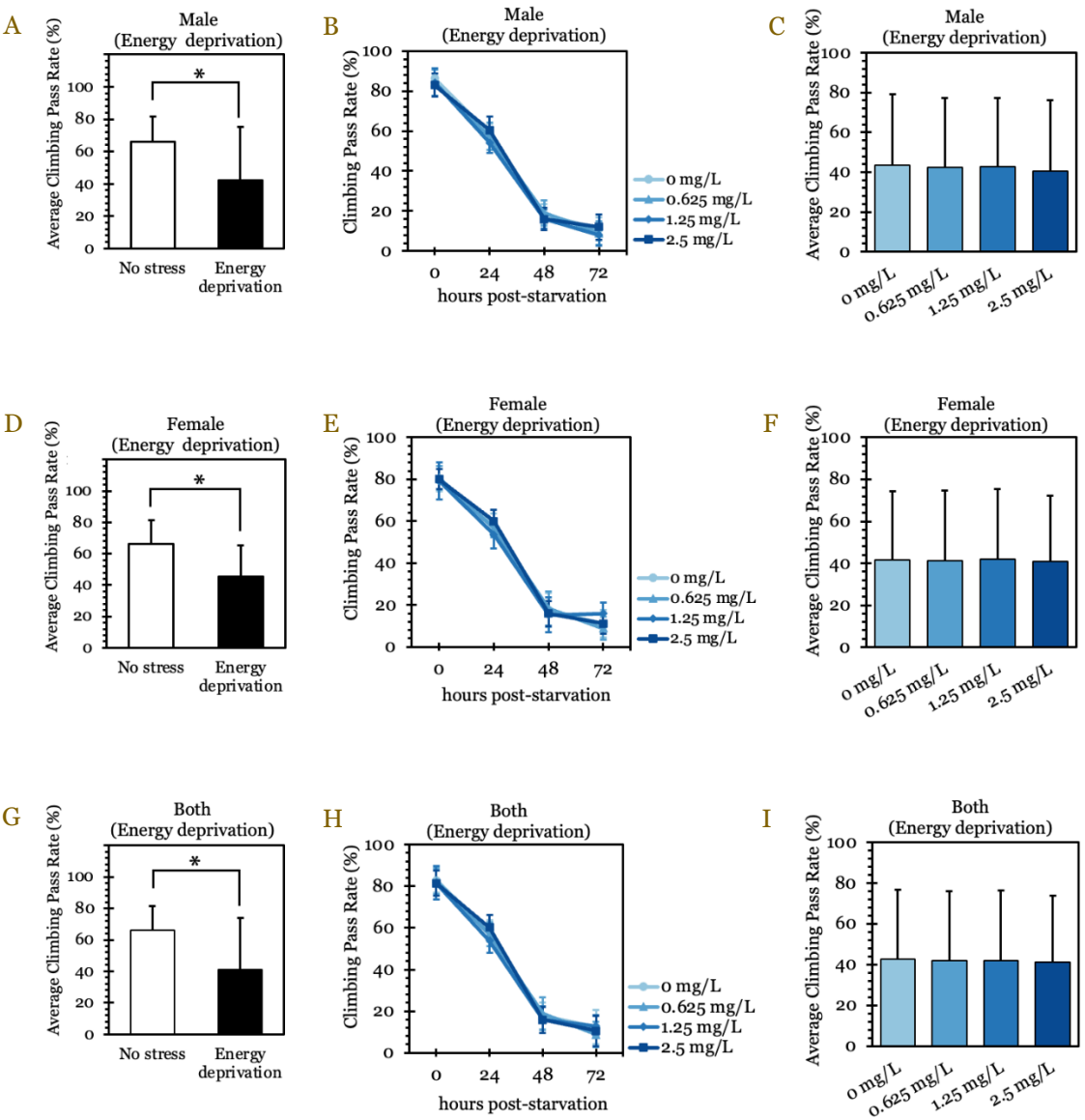

Figure 6. Acute sodium metabisulfite consumption does not affect the climbing activity of *D. melanogaster* under energy deprivation. Thirty male and thirty female flies were fed with varying concentrations (0, 0.625, 1.25, and 2.5 mg/L) of sodium metabisulfite in their diet for five days. Their diet along with the sodium metabisulfite was removed after the 5th day and the effect of energy deprivation on the climbing activity of the untreated male flies (A), daily climbing activity (B), and average climbing activity of male flies (C) was determined using negative geotaxis assay while the flies were under energy deprivation. The effect of energy deprivation on the climbing activity of the untreated female flies (D), daily climbing activity (E), and average climbing activity of female flies under energy deprivation (F) were evaluated using the same assay. The effects of energy deprivation on climbing activity (G), daily climbing activity (H), and average climbing activity of both male and female flies (I) were also assessed. *Mean±SD has a significant difference at $p<0.05$.

Similar to male flies, pre-treatment with sodium metabisulfite did not significantly alter the locomotor response of female flies to energy deprivation. Both treated and untreated female flies experienced a similar reduction in climbing ability (**Figure 6E** and **6F**). The observed reduction in climbing pass rate over time was not influenced by sodium metabisulfite exposure, suggesting that the compound does not negatively impact motor activity in female flies under these stressful conditions.

When both sexes were combined, their climbing activity was reduced by 37.8% ($p<0.05$) (**Figure 6G** and **7G**). A significant decline in climbing activity was observed after 72 hours,

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regardless of sodium metabisulfite treatment (**Figure 6H**). The average climbing activity with and without treatment was comparable (**Figure 6I**).

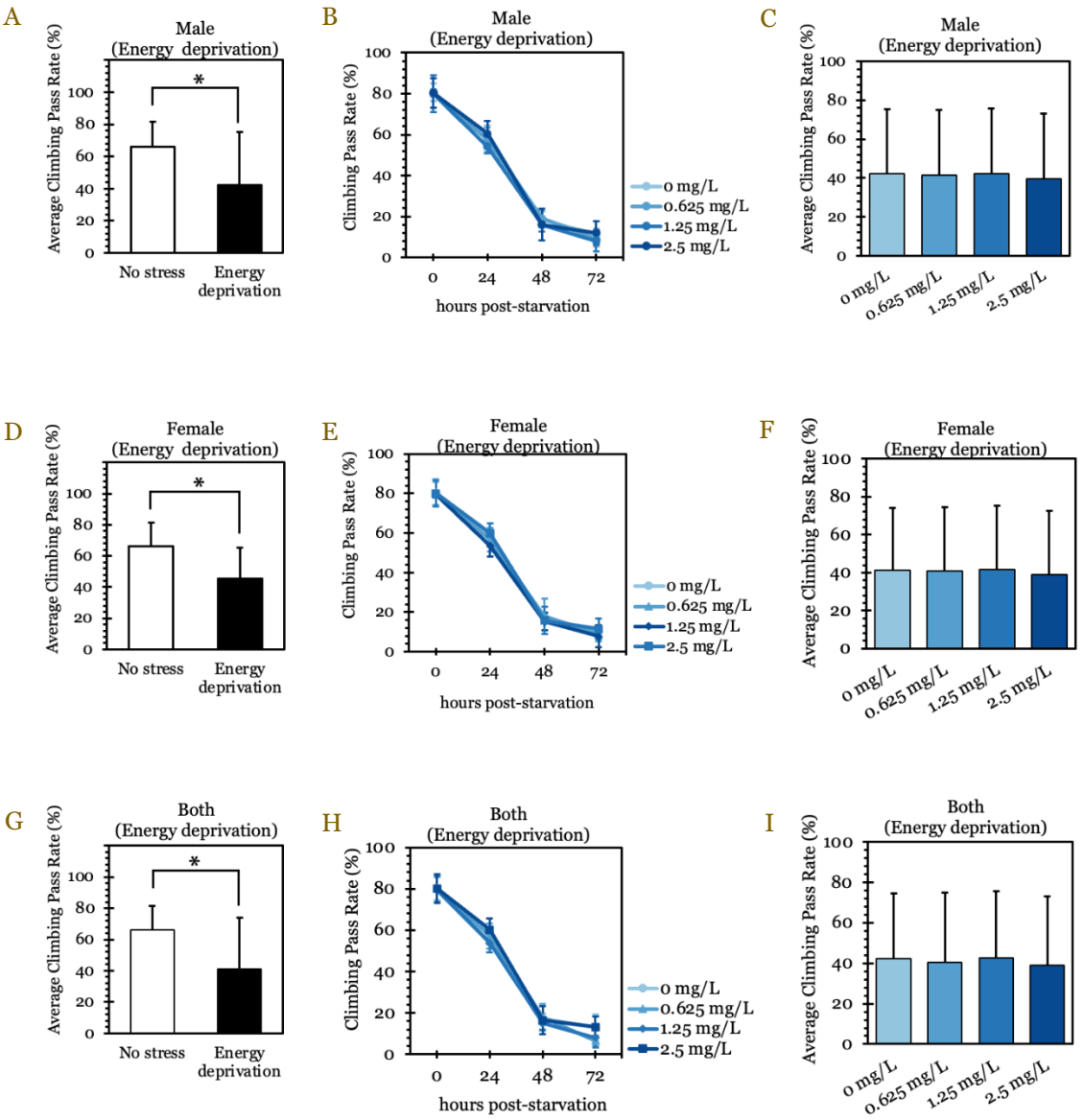

Figure 7. Chronic sodium metabisulfite consumption does not affect the climbing activity of *D. melanogaster* under energy deprivation. Thirty male and thirty female flies were fed with varying concentrations (0, 0.625, 1.25, and 2.5 mg/L) of sodium metabisulfite in their diet for five days. Only their diet was removed after the 5th day while sodium metabisulfite solution was constantly given to them. The effect of energy deprivation on the climbing activity of the untreated male flies (A), daily climbing activity (B), and average climbing activity of male flies (C) were determined using negative geotaxis assay while the flies were under energy deprivation. The effect of energy deprivation stress on the climbing activity of the untreated female flies (D), daily climbing activity (E), and average climbing activity of female flies under energy deprivation (F) were evaluated using the same assay. The effects of energy deprivation on climbing activity (G), daily climbing activity (H), and average climbing activity of both male and female flies (I) were also assessed. *Mean±SD has a significant difference at $p<0.05$.

Male flies administered various concentrations of sodium metabisulfite five days prior to and during the energy-deprived state exhibited climbing activity comparable to that of untreated control flies (**Figure 7B** and **7C**). While there was a general reduction in the climbing pass rate over time, this decline was consistent across all groups, indicating that sodium metabisulfite did not specifically impair the motor activity of the male flies under conditions of energy deprivation.

Female flies displayed a similar pattern (**Figure 7E**). Those fed with sodium metabisulfite five days before and during starvation showed climbing activity comparable to that of untreated flies (**Figure 7F**). The observed reduction in climbing pass rate over time was not influenced by sodium metabisulfite exposure, suggesting that the compound does not negatively impact motor activity in female flies under these stressful conditions.

A general decline in climbing performance after 72 hours was evident when the climbing activity of male and female flies were combined, regardless of treatment (**Figure 7H**). However, the average climbing activity between chronic sodium metabisulfite supplementation and untreated groups remained comparable (**Figure 7I**).

Discussion

Previous studies have indicated that sodium metabisulfite induced neurodegeneration in the hippocampus of rats [19,20]. The hippocampus is a crucial brain structure responsible for learning and memory in mammals, whereas mushroom bodies (corpora pedunculata) are essential brain structures for associating olfactory cues with rewarding or aversive outcomes in *Drosophila* [21,22]. Studies found that rats treated with sodium metabisulfite exhibited problems with learning and memory [23,24]. However, studies reported no changes in the cognition of rats treated with sulfites [19, 25,26]. Interestingly, another report suggested that sodium metabisulfite enhances short-term spatial learning and memory [27]. The concentrations of sodium metabisulfite used varied between those studies. The studies that observed the problems with learning and memory used concentrations ranging from 25 mg/kg (25 ppm), 100 mg/kg (100 ppm) to 260 mg/kg (100 to 260 ppm) [23,24]. The studies that did not observe cognitive impairment used doses of 500 mg/kg (500 ppm) and 25 mg/kg (25 ppm) [19,25,26], while the study reporting improved learning and memory employed a concentration of 100 mg/kg (100 ppm) [27]. Based on these varying concentrations, there is no definitive evidence that cognitive impairment is directly correlated with high levels of sodium metabisulfite.

In our study, we utilized concentrations of 0.625 to 2.5 mg/L (0.625 to 2.5 ppm), which are significantly lower than those previously reported. At these concentrations, no impairment or improvement in learning and memory was observed in *D. melanogaster*. This suggests that at these lower concentrations, the neurons in the corpora pedunculata of the *Drosophila* brain may be unaffected.

The motor cortex, basal ganglia, and cerebellum are the primary regions in the brain involved in various motor activities and the coordination of movement [28]. Studies have shown that the cerebral cortex, striatum in the basal ganglia, and cerebellum are vulnerable to sulfite toxicity [29-31]. Additionally, sulfites have been implicated in motor neuron toxicity, potentially contributing to amyotrophic lateral sclerosis [32]. For instance, sodium metabisulfite has been shown to reduce motor coordination in rodents and locomotion in zebrafish [30,33]. Conversely, another study reported increased motor activity in sulfite-treated rats [34], while yet another found no effect on motor activity [35].

Studies reporting negative effects of sulfites on motor activity used 25 mg/kg (25 ppm) of sodium metabisulfite in rats and 0.5 g/L (500 ppm) of sodium sulfite in zebrafish [30,33]. In contrast, rats that exhibited increased motor activity were administered 100 mg/kg (100 ppm) of sodium metabisulfite [34]. Similarly, no observed effects were reported with 100 mg/kg (100 ppm) of sodium metabisulfite [35]. The differing nature of sulfite compounds, such as sodium metabisulfite and sodium sulfite, may account for the variations in observed effects, despite their similar metabolic pathways. Both compounds are converted to sulfur dioxide and then sulfite ions in the body [36]. These sulfite ions are primarily oxidized into sulfate by the enzyme sulfite oxidase and excreted in urine, with minor pathways converting sulfite into thiosulfate or S-sulfonate compounds [36]. Notably, rats given lower amounts (25 ppm) of sodium metabisulfite demonstrated reduced motor activity compared to those given higher amounts (100 ppm). These data suggest that lower amounts of sodium metabisulfite may pose neurotoxic risks in rats, warranting further investigation.

In our study, *Drosophila* were treated with 0.6 to 2.5 mg/kg (0.6 to 2.5 ppm) of sodium metabisulfite, and no effect on their climbing activity was observed. In fruit flies, the central body complex is the primary brain region involved in motor function [37]. Our findings suggest that

low concentrations of sodium metabisulfite do not affect the neurons in the central body complex, as evidenced by the absence of changes in their climbing activity.

Studies have reported that thermal stress reduces general motor activity in rats and impairs motor cortical activity and complex motor performance in humans [38-40]. However, there is a lack of studies on the effects of sulfites on animals under heat stress. One study suggested that treating *Caenorhabditis elegans* with 0.5 to 2 g/L (500 to 2000 ppm) sodium sulfite increases the expression of hypoxia-inducible factor 1 (HIF-1), a response similar to that observed under heat stress [41]. HIF-1 has long been implicated as a protein target in motor neurodegeneration [42,43]. When we exposed fruit flies to heat stress, it may have activated HIF-1 proteins and accelerated senescence, particularly of the motor neurons, resulting in reduced motor activity. The overexpression of HIF-1 may be upended through the upregulation of TOR (target of rapamycin) protein or downregulation of Forkhead transcription factor (FOXO) [44]. Conversely, the concentrations of sodium metabisulfite (0.6 to 2.5 ppm) administered to the fruit flies may not have been sufficient to affect HIF-1, TOR, or FOXO. Consequently, these concentrations did not exacerbate the decline in motor activity observed in the fruit flies over time.

Studies have reported that UVA exposure leads to the potential deterioration of neural networks and pharyngeal muscles in *C. elegans* [45-46]. These studies indicated that UVA accelerates the aging process by increasing reactive oxygen species (ROS) levels and altering the DAF-16/FOXO and mitogen-activated protein kinase (MAPK) pathway, which is critical for regulating oxidative stress responses and DNA repair mechanisms [45-47]. This accelerated aging process likely contributes to the observed reduction in motor activity in *Drosophila* as a consequence of UVA exposure. To the best of our knowledge, there are no reported direct effects of sodium metabisulfite on multicellular organisms exposed to UVA radiation. The available studies on the effects of sulfites on oxidative stress have primarily been conducted on cultured cells, yielding mixed conclusions. Most studies suggest that sulfites induce oxidative stress in kidney cells, gastric mucosal cells, and erythrocytes [48-50]. Conversely, one study suggests that sulfites have protective effects against oxidative stress in neurons [51]. These studies used 5 to 500 µM (630 to 63,000 ppm) of sodium sulfite in kidney cells, 0.5 to 5 mM (63,000 to 630,000 ppm) of sodium sulfite in gastric mucosal cells, and 25 mg/kg (25 ppm) of sodium metabisulfite in rats [48-50]. The study demonstrating neuroprotection used 0.1 to 100 µM (12.6 to 12,600 ppm) of sodium sulfite [51]. These findings suggest that high concentrations of sulfites may induce oxidative damage, while lower concentrations may have antioxidant activity. In our experiment, the flies were fed with 0.625 to 2.5 ppm of sodium metabisulfite. These concentrations may not be sufficient to trigger oxidative stress or offer neuroprotection, which may explain the absence of protective or detrimental effects on motor activity under UVA stress.

Chronic energy deprivation leads to a reduction in skeletal muscle protein and intracellular signaling protein biosynthesis [52]. Mitochondria undergo adaptive biogenesis through AMP-activated protein kinase (AMPK) activation to prevent a drastic decline in muscle protein and intracellular signaling [53]. AMPK, a pivotal regulator of energy homeostasis, attenuates ATP-consuming anabolic processes while stimulating ATP-generating catabolic pathways [54,55]. AMPK is also responsible for various transcriptional regulatory activities of genes governing muscle fiber contractility and endurance [56]. Concurrently, the transcription factors forkhead in rhabdomyosarcoma (FKHR) and forkhead transcription factor like 1 (FKHRL1) are upregulated in murine skeletal muscle during starvation, inducing pyruvate dehydrogenase kinase 4 (PDK4) expression [57]. The subsequent recruitment of muscle atrophy F-box to myogenin by PDK4 culminates in myogenin ubiquitination and proteolysis [58]. To the best of our knowledge, there is still no evidence that links sodium metabisulfite consumption to the AMPK, FKHR, FKHRL1, or PDK4 signaling cascades. Therefore, further studies assessing the effect of metabisulfite on AMPK, FKHR, FKHRL1, or PDK4 signaling cascades are important.

Conclusion

The study investigated the effects of sublethal concentrations of sodium metabisulfite on cognitive function, motor activity, and stress tolerance in the fruit fly *D. melanogaster*. Exposure to sublethal doses of sodium metabisulfite did not produce any significant impairments in the cognitive and motor function of fruit flies. These findings suggest two possible interpretations:

(1) sodium metabisulfite may act through independent pathways that are not involved in cognition, muscle function, or oxidative stress responses in *D. melanogaster*, or (2) the concentrations used in this study were insufficient to elicit a detectable response. We recommend that future studies include a broader range of sodium metabisulfite doses alongside direct measurements of sulfite levels, ROS, and food intake to further assess dose-dependent effects on cognitive, motor function, and oxidative stress.

Ethics approval

This study involved the use of invertebrate animals, which are exempt from IACUC review at the University of the Philippines Manila.

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None.

Competing interests

The authors declare that there is no conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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