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Original Article
Curcumin Mitigates Mercury-induced Memory Impairment and Neurotoxicity: Insights from the *Drosophila melanogaster* Model

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ABSTRACT

Mercury-containing products, such as dental amalgam used for dental filling in dentistry, have been heavily criticised due to their associated toxicity. Nevertheless, it is still in use in low-income countries due to it being relatively affordable. Therefore, in this study, we evaluate the neurotoxic effects of mercury (Hg) and the possible ameliorative effects of polyphenol-curcumin using the fruit fly (*Drosophila melanogaster*) model. Male and female *D. melanogaster*, aged 3 to 5 days, were categorized into six groups, each consisting of 40 flies. To evaluate the impact of Hg exposure and the potential protective effects of curcumin, we conducted measurements on multiple parameters. These included assessing the survival rate, determining the memory index using an aversive phototaxis suppression assay, evaluating locomotor performance through negative geotaxis, and analysing various biochemical parameters associated with antioxidant status and neuronal function-related enzymes. Our findings indicate that dietary exposure to Hg resulted in a decreased survival rate and impaired locomotor performance in *D. melanogaster*. However, we observed a notable improvement in the antioxidant system among *D. melanogaster* exposed to Hg toxicity when they were fed a diet supplemented with curcumin. This improvement was evident through enhanced catalase activity, improved memory index, and modulation of acetylcholinesterase and monoamine oxidase activities. These effects were observed in comparison to *D. melanogaster* solely exposed to $HgCl_2$ without curcumin supplementation. Collectively, these data showed that curcumin could mitigate the neurotoxicity associated with Hg and thus could serve as a substitute for Hg-containing products like dental amalgam.

INTRODUCTION

Mercury (Hg) is a heavy metal that exists in a liquid state at room temperature. It is biologically non-functional and offers a significant risk of environmental pollution, particularly in aquatic environments where it spreads quickly down the food chain; it is regarded as a potentially hazardous agent to humans (Chen *et al*., 2009). Humans have mined, refined, and utilized Hg for quite a number of years, and despite the well-documented toxicity of these substances, exposure to them has persisted and even risen, primarily in developing nations (Järup, 2003). Additionally, human activities can unintentionally trigger the release of Hg from natural sources, contaminating water supplies and forcing creatures higher up the food chain to absorb it (Walters *et al*., 2011).

There have been concerns regarding the potential toxicity of Hg associated with dental amalgam utilisation as a dental filling material. Rare instances of allergic reactions have been documented in reported cases following the utilization of dental amalgam (Kal *et al*., 2008). In rodents

and human beings, high Hg levels are linked to ne-phrotoxicity, hepatotoxicity, and brain impairment (Mumtaz *et al*., 2019; Yadav *et al*., 2019; Zhang *et al*., 2023). It is present in the air, soil, and water, as well as the crust of the earth. The different chemical forms that this metal can take can be categorized as follows: inorganic Hg, primarily in salt form (HgS), elemental or metallic Hg, and its salt (HgCl$_2$). Exposure of rodents to both organic and inorganic Hg forms has been associated with psychological symptoms, irreversible destruction of the nervous system, kidney and immune system damage, and many metabolic diseases, such as cancer, circulatory, and heart diseases, among other pathologies (Zahir *et al*., 2005; Lu *et al*., 2017; Rossner *et al*., 2019).

While studies showed the vulnerability of invertebrates to chemical pollutants, such as heavy metals, it is notable that there are few studies addressing the effects of Hg on invertebrates (Posgai *et al*., 2011). This is despite the fact that there is quite a bit of literature on the impacts of Hg on vertebrates. The significant toxicity of Hg is closely associated with its ability to exacerbate oxidative stress, resulting in the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radical. This toxicity also results in the suppression of antioxidant enzyme and non-enzyme activity, impairing the protective defence mechanisms against oxidative damage. These factors have the potential to disrupt dopamine levels and give rise to considerable motor and neurological dysfunctions (Tamás and Zelinová, 2017). According to Franco *et al*. (2007), the increase in oxidative stress could be attributed to the diminishment of thiol compounds, primarily glutathione (GSH), a decrease in the expression of antioxidant enzymes, or a combination of both mechanisms. These events may harm cells, destroy biomolecules, and cause lipid peroxidation (Rudenko *et al*., 2021).

The fruit fly (*Drosophila melanogaster*) is one of the most prominent model organisms in contemporary genetics, with a rich literature covering classical and modern genetics, physiology, and biochemistry. Although *D. melanogaster* and humans are relatively distantly linked from an evolutionary perspective, functional counterparts of approximately 75% of genes associated with human diseases can be found in *D. melanogaster* (Pandey and Nichols, 2011). This characteristic renders *D. melanogaster* an appropriate model for studying humans and other vertebrates. *D. melanogaster* is one of the ideal organisms to employ in in vivo bioassays since they have a quick reproduction cycle and are simple to maintain and handle in the laboratory (Panchal and Tiwari, 2017). They are an effective model system for research into the anatomy and physiology of the nervous system as well as the basic cellular mechanisms underlying metal and pesticide toxicity (Bonilla-Ramirez *et al*., 2011).

The natural polyphenol molecule curcumin, or 1,7-bis-[hydroxy-3-methoxyphenyl]-1,6-heptadiena-3,5-dione (C21H20O6), has a molecular weight of 368.38 g/mol. It is turmeric's major bioactive component. According to Sharma et al. (2005), the yellowish chemical curcumin exhibits potent biological characteristics, encompassing anti-

inflammatory, antiviral, anticancer, antioxidant, and antibacterial properties. Curcumin, and its derivatives (Mythri *et al*., 2011), micronized (Sandhir *et al*., 2014), hybridised (Chojnacki *et al*., 2014), and synergistic combination with other compounds (Banji *et al.*, 2014) have demonstrated to possess a remarkable ability to intracellularly neutralise reactive nitrogen species (RNS) as well as ROS (Mishra *et al*., 2004). Additionally, scientific evidence supports the pharmacological impact of curcumin in retarding the ageing process and extending the lifespan of *D. melanogaster*, although the mechanism is yet unknown (Salvioli *et al*., 2007; Lee *et al*., 2010; Sikora *et al*., 2010). In two separate wild-type *D. melanogaster* strains, Lee et al. (2010) revealed that curcumin protected against oxidative stress, increased feeding rate, life span, and modified gene expression. Also, in a *D. melanogaster* model, curcumin has been demonstrated to enhance antioxidant enzymes and modulate the expression level of acetylcholinesterase genes (Akinyemi *et al*., 2018).

Considering the involvement of free radicals in the advancement of neurodegenerative disorders and the significance of employing effective antioxidants for prevention and treatment, we hypothesize that investigating the potential of curcumin as a neuroprotective agent and as a regulator of the impact of Hg chloride in the *D. melanogaster* model shows promise in mitigating the harmful effects of Hg. Therefore, in the current study, we report the biochemical responses of *D. melanogaster* to Hg chloride (HgCl$_2$) exposure and the positive impact of curcumin on Hg toxicity, with a focus on biomarkers of oxidative stress and neurodegeneration.

MATERIALS AND METHODS

Materials
Sample Collection
The *D. melanogaster* (Oregon strain) stock used in this study was acquired from the Drosophila research laboratory at the Functional Food and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology in Akure, Nigeria. Hg chloride (CAS 7487-94-7) was sourced from Fisher ScientificTM, UK.

Reagents
All utilized reagents were sourced from reputable suppliers and were of standard grade, while the water used was distilled using glass equipment.

Methods
D. melanogaster Culture
D. melanogaster (Oregon strain) were cultured and raised on a standard diet comprising 12% agar, 79% corn flour, 0.5% yeast, and 1.5% nipagin. The culture conditions were maintained at a constant temperature of 25±1°C and a relative humidity of 60%. Female *D. melanogaster* were given a period of 3-4 days to lay eggs, after which the older *D. melanogaster* were transferred to allow for the emergence of a new generation. The newly emerged *D.*

melanogaster were allowed to mature for 3-5 days before the process was repeated to obtain a sufficient number of flies for the experiment. Throughout the study, the same strain of *D. melanogaster* was used consistently. All applicable institutional ethics for laboratory studies were followed.

Ethical Approval
The use of *D. melanogaster* for laboratory research is not subject to ethical approval. Nevertheless, the research was carried out in adherence to the ethical guide for laboratory experimental research at the Federal University of Technology, Akure, Nigeria.

Experimental Design
Three to four-day-old *D. melanogaster* were divided into six groups, with each group consisting of six vials containing 40 flies per vial. The *D. melanogaster* in each group were subjected to different diets and treatments, as outlined below:
Basal Diet (I)
Basal diet +1.0 mM $HgCl_2$ (II)
Base diet + 2.0 mM $HgCl_2$ (III)
Basal diet + curcumin (2.7 mM) (IV)
Base diet + curcumin + 1 mM $HgCl_2$ (V)
Basal diet + curcumin + 2 mM chloride (VI)

The choice of doses of $HgCl_2$ chloride (1 mM and 2 mM) was based on our preliminary study (data not shown) on the mechanism of Hg neurotoxicity at different doses of exposure, as well as our previous study (Ogunsuyi *et al*., 2022). Translationally, this will represent lower and higher exposures to $HgCl_2$ chloride. The choice of dose of curcumin was based on our previous study, in which curcumin showed neuroprotective properties at this dose equivalent (Akinyemi *et al*., 2018).

Survival Study
An experiment was conducted to evaluate the impact of different concentrations of $HgCl_2$ and curcumin in the diet on the survival rate of *D. melanogaster* over a period of seven days. *D. melanogaster* that were 3–5 days old were divided into six groups, each consisting of six vials, and were exposed to different concentrations of $HgCl_2$ and curcumin. The *D. melanogaster* were closely monitored for seven days to record any instances of mortality, and the survival rate was determined by counting the number of deceased flies during this period. The collected data were analysed and presented graphically as cumulative mortality and the percentage of surviving *D. melanogaster* after the treatment duration.

Measurement of Locomotor Performance by Negative Geotaxis Assay
The assessment of locomotor performance in both the control and treated groups of *D. melanogaster* was conducted on the last day of the experiment (day 7), employing the negative geotaxis assay as previously reported (Le Bourg and Lints, 1992). After treatment, the surviving flies from the experimental groups were

individually immobilised by placing them in a labelled, sterilised tube measuring 11 cm in length and 3.5 cm in diameter. Following a recovery period of 10 min, the *D. melanogaster* were softly tapped at the base of the tube, and the count of flies that successfully crossed the 6 cm mark within 30 s was recorded. Typically, *D. melanogaster* without any locomotor impairments exhibit rapid movement towards the top of the tube, while those with motor defects exhibit slower movement or may remain near the bottom. The climbing scores represent the average percentage of *D. melanogaster* that successfully crossed the 6 cm mark out of the total number of flies in each experiment. The results are expressed as the percentage of *D. melanogaster* that managed to surpass a minimum distance of 6 cm within 30 s, based on three trials.

Memory Retention Assay
The impact of associating an odorant with pain perception was assessed on the last day of the experiment (day 7), following the established methodology described in previous studies (Zars *et al*., 2000; Zamberlan *et al*., 2020). The *D. melanogaster* were trained in a chamber where they were exposed to 3-octanol (OCT, 1:100) paired with electrical shocks (75 V) for a duration of 4 min, while methylcyclohexane (MCH, 1:25) was presented without any accompanying electrical shocks. Following the training phase, the *D. melanogaster* were returned to their regular diet for a period of 10 min. Subsequently, a memory test was conducted. The *D. melanogaster* were placed at a choice point between converging air currents infused with the two odours (OCT and MCH), and they were allowed to move towards either the OCT or MCH chamber. The odours were delivered into the respective chambers through an airflow system. The performance index, used as a measure of memory, was calculated by dividing the count of *D. melanogaster* within the OCT chamber by the overall number of flies and expressed as a percentage of the overall number of flies.

D. melanogaster Tissue Homogenate Preparation
D. melanogaster were rendered immobile by exposure to ice and swiftly decapitated. The heads and the body (n = 5) were then subjected to homogenization separately in 0.1 M potassium phosphate buffer with a pH of 7.4. The homogenates were subjected to centrifugation at 4 °C and 13,000 rpm for ten minutes. The protein contents of the resulting homogenates were quantified using Nanodrop2000™ and adjusted to a concentration of 0.4 mg/mL. The homogenates were utilized to measure the levels of ROS, total thiol contents, and catalase activity.

Measurement of 2',7'-dichlorodihydrofluorescein diacetate (DCFH) Fluorescence
The reaction mixture consisted of 75 mM potassium phosphate buffer with a pH of 7.4, 5 μM 2',7'-dichlorodihydrofluorescein diacetate (DCFH), and 5 μL of the homogenate. Subsequently, the fluorescence emission was measured using a Spectra Max spectrofluorimeter plate reader for a duration of 30 min, with readings taken every thirty seconds (excitation = 480 nm; emission = 525

nm). The results were expressed as the change in fluorescence intensity per minute, following the methodology described in previous studies (Pérez-Severiano *et al*., 2004; Afolabi and Olagoke, 2020).

Determination of the Total Thiol Content
The total thiol content in the *D. melanogaster* head and body homogenate was assessed employing the method developed by Ellman (1959). To carry out this determination, a reaction mixture was prepared, consisting of 200 μL in total volume. The mixture included 85 mM potassium phosphate buffer (pH 7.4), 20 μL of tissue homogenate, and 0.5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). In order to create reaction blanks, the same amount of homogenate was added to the mixture, but without DTNB. Subsequently, the resultant mixture was incubated at room temperature for 30 min, followed by measuring the absorbance at 412 nm. Subsequently, the total thiol content was calculated and expressed as (μmol/mg protein).

Catalase (CAT) Activity Assay
The activity of CAT was determined following the method developed by Aebi (1984). The reaction mixture consisted of 1 mL in total volume, containing 40 mM potassium phosphate buffer (pH 7.0), 14.1 mM H_2O_2, and 20 μL of tissue homogenate. The CAT activity was evaluated through the measurement of the amount of H_2O_2 consumed at 25 °C. The decrease in H_2O_2 was monitored over a duration of 2 min, with measurements taken every 10 s, using a UV-visible spectrophotometer at a wavelength of 240 nm. The results were expressed as mmol of H_2O_2 consumed per minute per milligram of protein.

Acetylcholinesterase (AChE) Activity Assay
The activity of AChE was measured using the Ellman method, as described by Ellman et al. (1961). The reaction mixture consisted of 10 mM potassium phosphate buffer with a pH of 7.4, 1.0 mM DTNB, 30 μL of homogenate, and 0.8 mM acetylthiocholine iodide. The AChE activities were monitored at a wavelength of 412 nm and were expressed as micromoles of acetylthiocholine (AcSch) hydrolyzed per hour per milligram of protein.

Monoamine Oxidase (MAO) Activity Assay
MAO activity was assessed following the methods outlined in previous studies by Green and Haughton (1961) and Turski et al. (1973), with minor adjustments. The reaction mixture comprised a phosphate buffer (0.025 M, pH 7.0), semicarbazide (0.0125 M), benzylamine (10 mM), and a homogenate of *D. melanogaster*. The measurement of the intensity of the resulting orange–yellow coloration was conducted at 280 nm.

Data Analysis
GraphPad PRISM (V.5.0) software was used for data analysis. The experimental results were presented as the mean ± standard error of the mean (SEM). Mean values were assessed for statistical significance using a two-way ANOVA to examine interactions among the different treat-

ment groups. Tukey's post-hoc test was conducted for further analysis. The level of significance was set at $p < 0.05$.

RESULTS

In this study, each *D. melanogaster* was subjected to Hg toxicity for 7 days. Two concentrations (1 mM and 2 mM) of $HgCl_2$ combined with curcumin were utilized. Survival rates, locomotor activity, memory index, and various biochemical parameters were measured.

First, the impact of curcumin-supplemented diets on the survival rate of *D. melanogaster* exposed to $HgCl_2$, along with simultaneous curcumin treatment was studied. According to the results shown in Figure 1, the survival rate of the *D. melanogaster* exposed to 1 mM and 2 mM Hg decreased significantly ($p < 0.05$) when compared to the control. Notably, the survival rate of *D. melanogaster* exposed to curcumin was not significantly different compared to that of the control. However, when compared to *D. melanogaster* exposed to $HgCl_2$ alone (1 mM and 2 mM), the amelioration in the reduced survival rate observed in flies exposed to curcumin + $HgCl_2$ (1 mM and 2 mM) was not statistically significant at $p<0.05$.
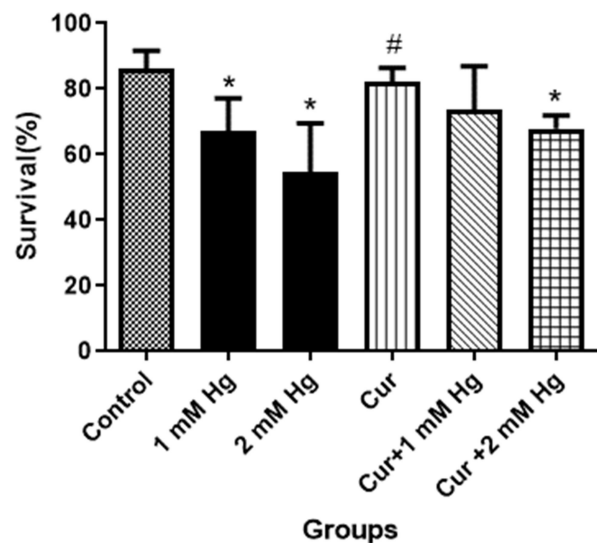

Fig. 1: Survival Rate (%) of *D. melanogaster* induced with $HgCl_2$ and co-treated with curcumin at 7 days post-treatment. Values represent the mean ± SD. * statistically significant difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group

Additionally, the impact of treatment on the memory index of *D. melanogaster* was evaluated using the aversive phototaxis suppression assay. The outcome of this analysis indicated that there was no significant difference in memory index between control and curcumin-fed *D. melanogaster*. Nevertheless, a significant ($p < 0.05$) decline in memory function was observed among *D. melanogaster* treated with 1 mM and 2 mM Hg in

comparison to the control group. However, in Hg-exposed *D. melanogaster* (1 mM and 2 mM) fed with curcumin-supplemented diets, a noteworthy enhancement in memory functions was observed when compared to flies exposed to Hg alone (Fig. 2).
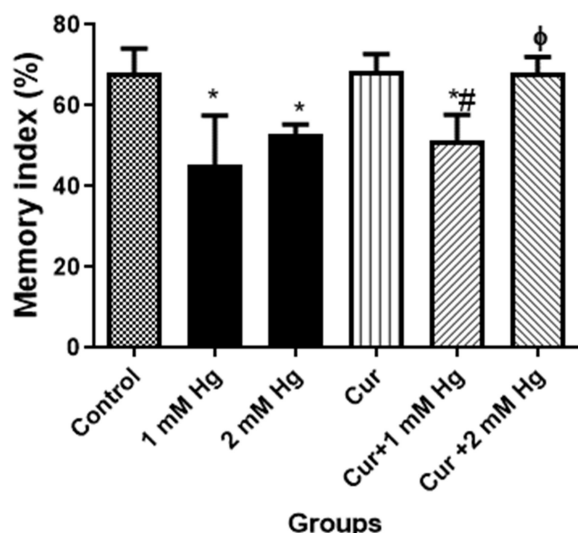

Fig. 2: Memory index (%) of D. melanogaster induced with mercury chloride and co-treatment with Curcumin at 7 days post-treatment. Values represent the mean ± SD; * statistically significant difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group
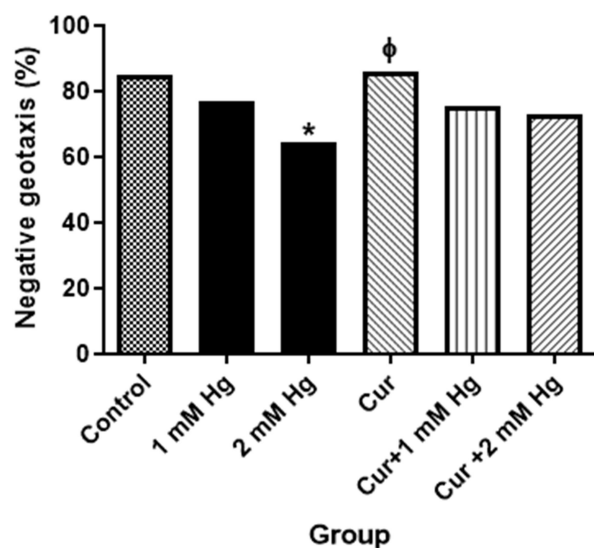

Fig. 3: Negative geotaxis of the induced HgCl₂ *D. melanogaster* co-treated with curcumin at 7 days post treatment. Values represent the mean ± SD; * statistically significant difference in mean values compared to the control group; φ statistically significant difference in mean values compared to 2 mM Hg

With respect to locomotor assessment, *D. melanogaster* exposed to 2 mM Hg exhibited a notable decrease in lo-

comotor performance and also took a longer time to traverse the 6 cm line within a 30-s timeframe. A significant number of flies remained at the base of the vial even with a tap to the base of the tubes, unlike the control group. However, no significant difference was observed between negative geotaxis in the control and curcumin treated groups. There was also no significant difference between *D. melanogaster* treated with $HgCl_2$ alone and those treated with $HgCl_2$ and curcumin (Fig. 3).

As a general measure of oxidative stress, the oxidation of DCFH was quantified, which reflects the level of ROS, as depicted in Figure 4. The results demonstrate a significant ($p < 0.05$) elevation in ROS levels in the head and body of *D. melanogaster* exposed solely to Hg in comparison to the control group. However, in both the head and body tissues, the elevated ROS levels were significantly ameliorated in Hg-treated *D. melanogaster* fed the dietary inclusion of curcumin, when compared to Hg-treated flies. No significant difference was, however, observed between the control group and curcumin-fed *D. melanogaster*.

The levels of thiobarbituric acid reactive substances (TBARS) in head and body tissues of *D. melanogaster*, to indicate lipid peroxidation was evaluated and the results shown in Figure 5. The level of TBARS was significantly increased ($p < 0.05$) in *D. melanogaster* treated with 1 mM and 2 mM Hg in comparison to the control flies. However, the administration of curcumin led to a significant ($p < 0.05$) reduction in the TBARS level in Hg-exposed *D. melanogaster* head (Fig. 5a). Similarly, there was a significant ($p < 0.05$) decrease in TBARS level in *D. melanogaster* body treated with 1 mM Hg and fed curcumin compared to flies treated with 1 mM Hg alone (Fig. 5b). Nevertheless, there was no significant difference in TBARS content between the control group and *D. melanogaster* fed curcumin alone.

The total thiol level as an indirect measure of antioxidant status is as presented in Figure 6. There was a notable decrease ($p < 0.05$) in the total thiol level in the head of *D. melanogaster* treated with 1 mM and 2 mM Hg compared to the control flies. A significant ($p < 0.05$) elevation was observed in Hg-exposed *D. melanogaster* treated with curcumin in comparison to those exposed to Hg alone (Figure 6a). However, the significant rise in the total thiol level was only observed in *D. melanogaster* treated with 2 mM Hg. Nevertheless, there was no significant difference in the total thiol content between the control group and *D. melanogaster* fed curcumin alone.

The CAT activity to assess oxidative status in the head and body of *D. melanogaster* is as depicted in Figure 7. The results indicate a significant ($p < 0.05$) decrease in CAT activity in *D. melanogaster* treated with Hg in comparison to the control flies. However, a significant ($p < 0.05$) increase in CAT activity was observed in *D. melanogaster* exposed to Hg but fed with curcumin when compared with those exposed solely to Hg. Nevertheless, there was no significant difference in CAT activity between the control group and *D. melanogaster* fed curcumin alone. The activities of AChE and MAO in the head and body of *D. melanogaster* were analysed as presented in Figures 8-9. The findings demonstrated that for AChE activity, a dual

effect was observed in the head of *D. melanogaster*, in which a significant reduction was observed at 1 mM Hg and a significant elevation was observed at 2 mM Hg. However, in the *D. melanogaster* body, both doses of Hg exposure caused a significant reduction in AChE activity.
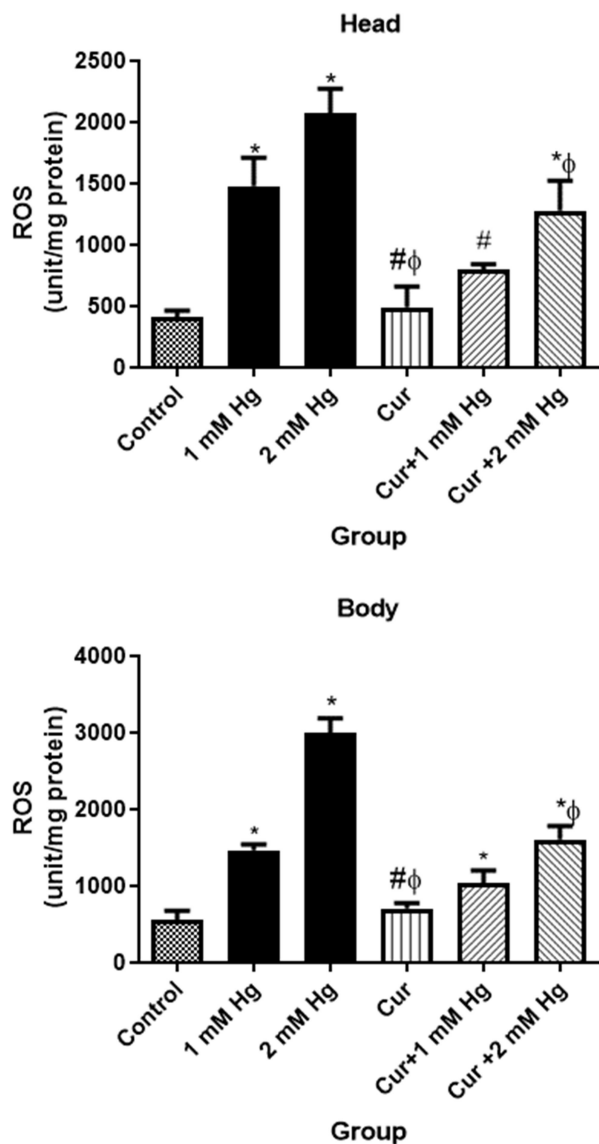
to Hg alone. But, there was no significant difference in both AChE and MAO 'activities between the control group and *D. melanogaster* fed curcumin alone.
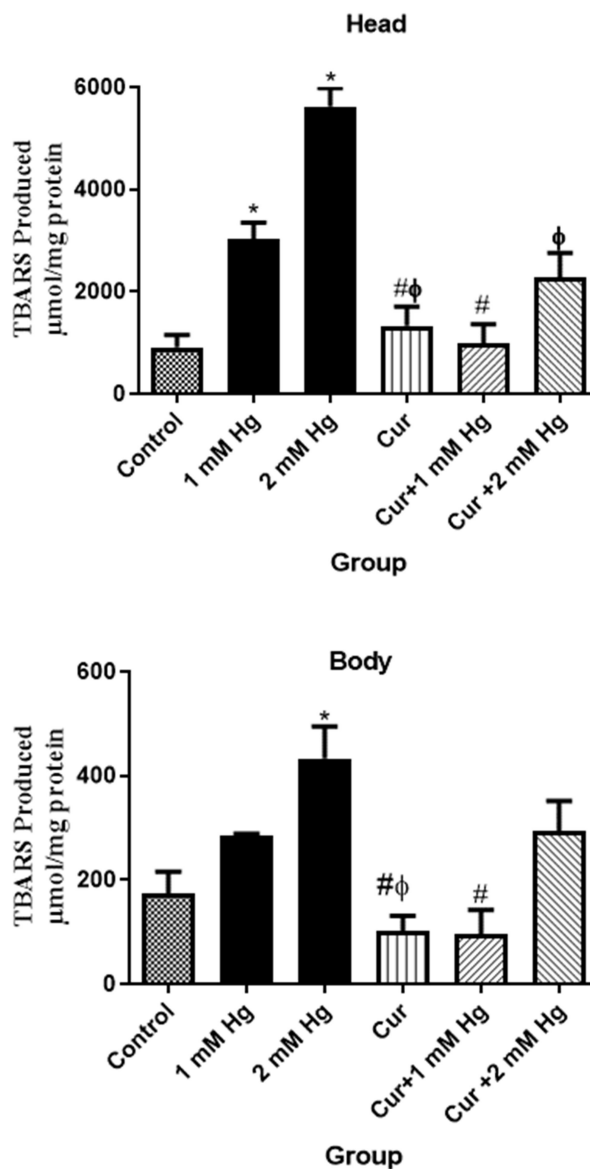

Fig. 4: ROS activity of HgCl$_2$ in *D. melanogaster* co-treated with curcumin at 7-days post-treatment. Values represent the mean ± SD; * statistically significant difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group

Fig. 5: TBARS produced in HgCl$_2$ -induced *D. melanogaster* co-treated with curcumin at 7 days post-treatment. Values represent the mean ± SD; * statistically significant difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group

Nevertheless, these impairments were ameliorated in Hg-treated *D. melanogaster* fed diets supplemented with curcumin. Furthermore, there was a significant ($p < 0.05$) increase in MAO activities in *D. melanogaster* exposed to Hg alone (2 mM Hg). However, a significant ($p < 0.05$) reduction in MAO activities was observed in Hg-exposed *D. melanogaster* fed curcumin compared to those exposed

DISCUSSION

Investigating diverse systems, including invertebrates and vertebrates, has expanded and enhanced our comprehension of the molecular processes involved in the toxicity of chemicals. This has resulted in advancements in our un-

derstanding of vulnerability and specific molecular objectives. Hg-containing products, such as dental amalgam used for dental filling in dentistry, have been heavily criticised due to their associated toxicity. Nevertheless, its use persists, especially in some developing and mostly underdeveloped countries (Arotiba *et al*., 2020). Consequently, this research contributes to the expansion and clarification of our understanding regarding the cellular targets of Hg in organisms, using the *D. melanogaster* model. Elevated levels of Hg have been associated with liver damage, neurological impairments, and kidney damage in various experimental models (Haouem *et al*., 2013; García-Niño and Pedraza-Chaverrí, 2014; Carrizalez-Yáñez *et al*., 2022; de Paula Arrifano *et al*., 2023). Research conducted in areas contaminated with

statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group

Hg has shed light on its toxic effects on wildlife, revealing a range of consequences such as respiratory disruptions in shrimp larvae, as well as harmful effects on the development of fish, birds, and mammals, including embryonic toxicity and birth defects (Ramírez-Rochín *et al*., 2021). In a recent study by Krout *et al*. (2022), it was revealed that *D. melanogaster* displayed vulnerability to exposure to methylmercury. Prior research has indicated that exposing *D. melanogaster* to copper (Cu), manganese (Mn), and iron (Fe) leads to compromised locomotor abilities. These deficits were linked to the degeneration of neurons and were observed after longer treatment durations (Saraiva *et al*., 2018).

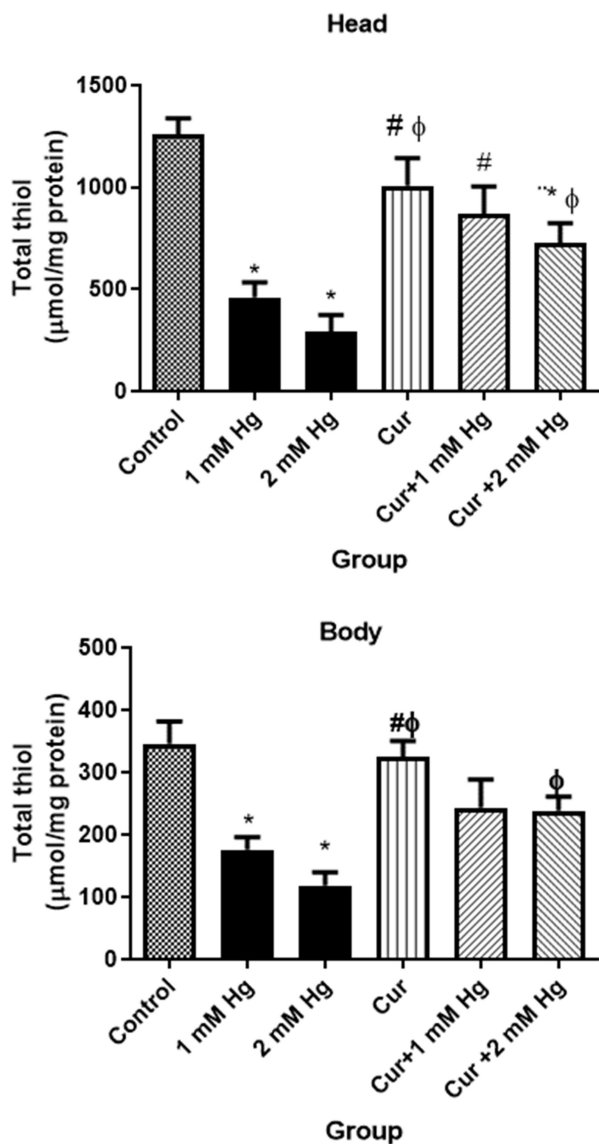Fig. 6: Total thiol activity of Hg-induced *D. melanogaster* co-treated with curcumin at 7 days post-treatment. Values represent the mean ± SD; * statistically significant difference in mean values compared to the control group; #

Fig. 7: Catalase activity of $HgCl_2$-induced *D. melanogaster* co-treated with curcumin at 7 days post-treatment. Values represent the mean ± SD; * statistically significant

difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group

Head

Body

Fig. 8: Acetylcholinesterase activity of *D. melanogaster* exposed to HgCl$_2$ at 7 days post-treatment. Values represent the mean ± SD. * statistically significan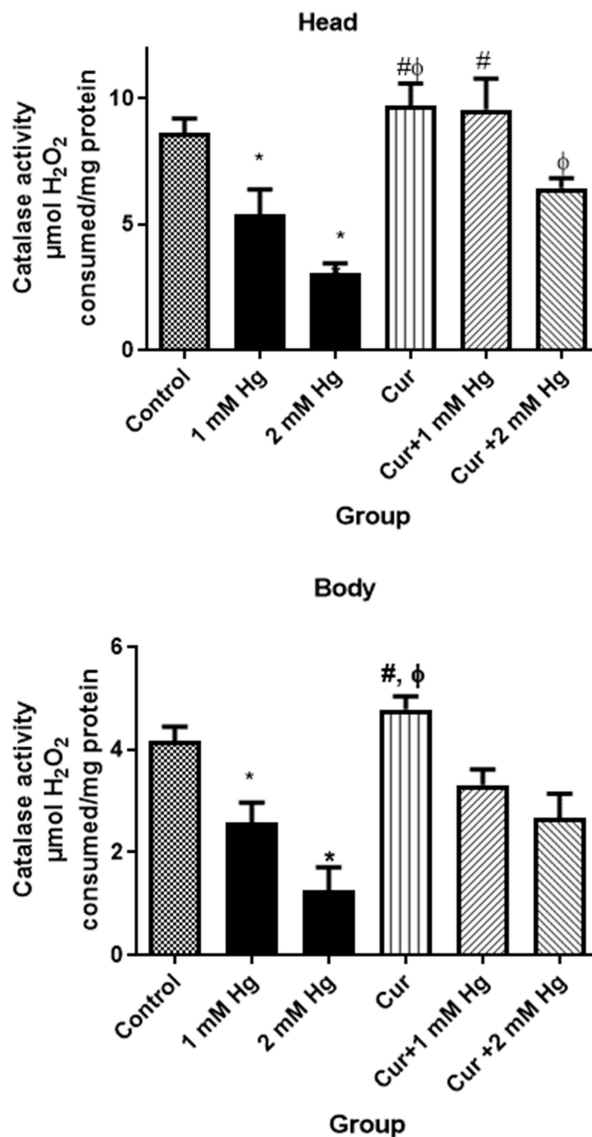t difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group
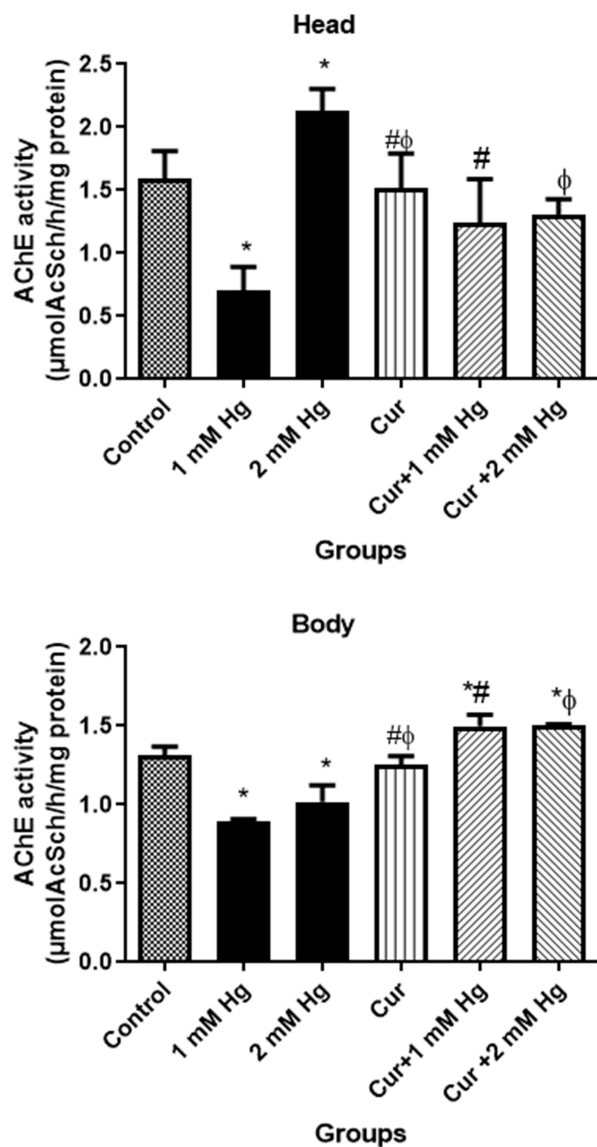
The results of this study demonstrated that *D. melanogaster* subjected to Hg toxicity and fed diets supplemented with curcumin exhibited a notable increase in survival rate compared to the flies fed solely Hg. Furthermore, these curcumin-supplemented *D. melanogaster* displayed improvements in locomotor performance, as well as memory index, along with a

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diminution in the activities of AChE and MAO. These outcomes suggest that curcumin, particularly in terms of memory function and cholinergic and monoaminergic neurotransmission, possesses significant neuromodulatory properties. In recent times, there has been growing interest in functional foods and nutraceuticals as promising agents possessing anti-ageing and neuroprotective attributes.

Head

Body

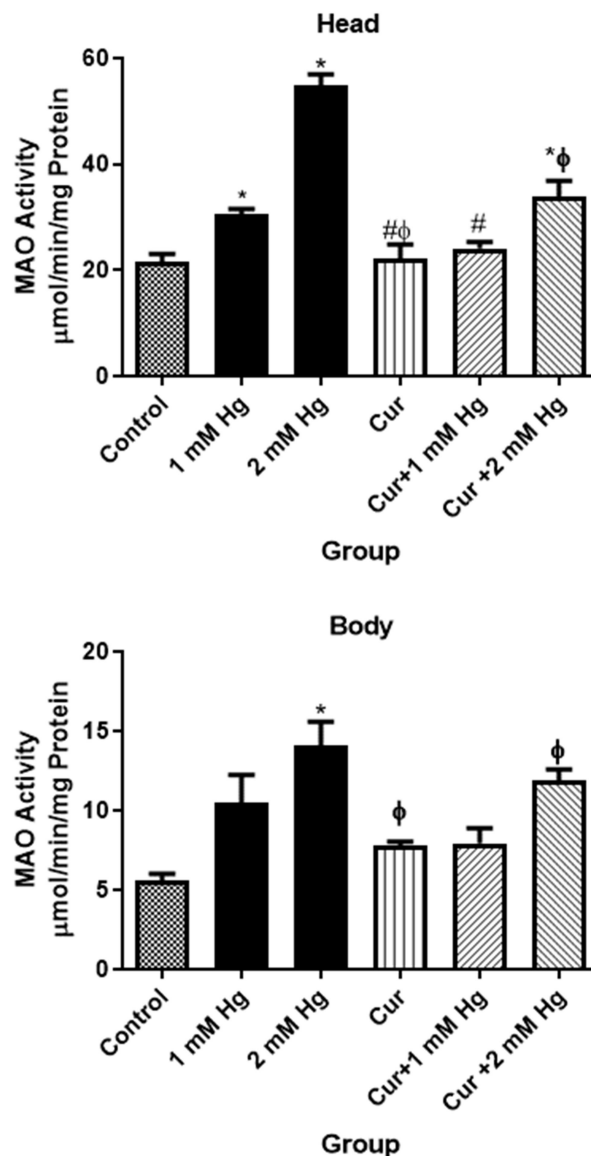Fig. 9: Monoamine oxidase (MAO) activity of HgCl$_2$-induced *D. melanogaster* co-treated with curcumin at 7 days post-treatment. Values represent the mean ± SD. * statistically significant difference in mean values compared to the control group; # statistically significant difference in mean values compared to the 1 mM Hg group; φ statistically significant difference in mean values compared to the 2 mM Hg group.

These neuroprotective potentials were associated with diverse processes, such as antioxidative effects and modulation of enzymes (Atlante *et al*., 2020; Ramli *et al*., 2020), leading to their significant recognition. Hence, in order to acquire a deeper grasp of the underlying processes accountable for the enhancements in behavioural performance, alongside the improved survival rate seen in *D. melanogaster* fed curcumin-supplemented diets, we investigated different biochemical markers related to toxicity and neuronal function.

Oxidative assault has been strongly associated with neurodegeneration and accelerated ageing (Ionescu-Tucker and Cotman, 2021). Disturbances in the oxidation-reduction balance within neurons have been suggested to trigger diverse molecular mechanisms, viz neuroinflammation, oxidative stress, and apoptosis, ultimately leading to neurodegeneration and accelerated ageing (Goldsteins *et al*., 2022; Hussain *et al*., 2022). The outcomes of our study are particularly significant, as they reveal that Hg exposure impaired the activity of antioxidant enzymes, led to excessive production of ROS, and promoted the peroxidation of lipids in *D. melanogaster*. These observations highlight the association between metal-induced neurotoxicity and oxidative stress, characterised by an abundance of ROS. During this investigation, we observed that *D. melanogaster* exposed to Hg toxicity and fed with diets supplemented with curcumin displayed a decrease in the generation of ROS and TBARS. Neurodegenerative processes linked to oxidative stress involve a series of biochemical events triggered by an elevation in free radicals (Singh *et al*., 2019). The heightened liberation of ROS is responsible for the oxidative destruction of various biomolecules, leading to carboxylation of proteins, destruction of genetic materials (specifically, DNA), and peroxidation of lipids. Consequently, an increase in TBARS production, a marker indicating peroxidation of lipids, further indicates an augmented level of ROS liberation and oxidative assaults. The decreased ROS and lipid peroxidation levels noticed in *D. melanogaster* exposed to Hg toxicity and fed diets containing curcumin signify the antioxidant properties of curcumin, which can be linked to their extended survival rate. These findings align with previous studies in *D. melanogaster*, demonstrating that curcumin reduces neurotoxicity in transgenic Drosophila (Caesar *et al*., 2012). Additionally, we noted a clear correlation between the reduction in ROS and TBARS levels in *D. melanogaster* subjected to Hg toxicity. This correlation was observed when the flies were provided with diets enriched with curcumin. Furthermore, there was an increase in the overall thiol levels in both the head and body of the *D. melanogaster*. Thiols, characterized by their sulfhydryl group, inherently possess antioxidant properties, contributing to their ability to reduce substances (Ulrich and Jakob, 2019).

Typically, thiols are involved in various redox reactions, including the conjugation of xenobiotics, and the neutralisation of free radicals, along with the reduction of ROS (Ulrich and Jakob, 2019). Furthermore, a decline in neuronal thiol content has been associated with ageing and neurodegenerative diseases, where a reduced level of thiols is linked to an increased rate of neuronal death (Cahill-Smith and Li, 2014). Thus, an elevation in the level of total thiol implies an enhancement in the flies' antioxidant capabilities, which may enhance their survival rate. Additionally, our study demonstrated a reduction in catalase activity exclusively in *D. melanogaster* exposed to Hg without any dietary supplementation. However, in Hg toxicity-induced flies that were fed diets supplemented with curcumin, a rise in catalase activity was noticed. Despite a substantial rise in catalase activity being detected in the heads of Hg toxicity-induced *D. melanogaster* fed with curcumin-supplemented diets, there was a non-significant increase in the bodies of these flies. Catalase is an endogenous enzyme responsible for converting hydrogen peroxide into water (Mahomoodally and MA-L, 2022). Hydrogen peroxide plays a significant role as a source of reactive species, which can cause various oxidative damages at the cellular level (Niedzielska *et al*., 2016). Significantly, in *D. melanogaster* subjected to Hg toxicity and provided with curcumin-containing diets, the observed rise in catalase activity corresponds with a decrease in the production of ROS and TBARS. This suggests a potential antioxidant mechanism. These findings are consistent with recent research emphasising curcumin's neuroprotective and mitochondria-protective attributes. They also support earlier studies where nutraceuticals promoting survival rates led to an increase in catalase activity (Bahadorani and Hilliker, 2008). Therefore, this study proposes that the reduction in ROS release observed in *D. melanogaster* exposed to Hg toxicity and fed curcumin-supplemented diets may be attributed to curcumin's antioxidant properties.

Alongside compromised redox signalling, disrupted neurotransmitter-mediated neuronal processes have also been linked to accelerated ageing and related neurodegenerative conditions (Reale and Costantini, 2021). Cholinesterase inhibitors, the primary symptomatic therapy for neurodegenerative diseases like Alzheimer's disease, function by inhibiting the enzymes responsible for the breakdown of acetylcholine in the synaptic cleft. This action is aimed at elevating the active levels of acetylcholine and is considered the first-line treatment for managing symptoms (Breijyeh and Karaman, 2020). Acetylcholine (ACh), a neurotransmitter in the cholinergic system, has a pivotal role in modulating various cholinergic functions, including memory, locomotor activities, and learning. AChE, a serine protease, breaks down acetylcholine into choline and acetate, thereby influencing cholinergic neurotransmission (Craig *et al*., 2011). This study revealed a significant reduction in the activities of AChE and MAO in *D. melanogaster* exposed to Hg toxicity and fed diets supplemented with curcumin, compared to those exposed to Hg alone. This implies that curcumin may possess therapeutic potential by inhibiting AChE activity, preventing the hydrolysis and breakdown of acetylcholine. The observed changes in AChE and MAO activities align with the increased levels of thiols, enhanced antioxidant enzyme activities, and decreased indicators of oxidative stress. The elevated AChE and MAO activities were observed in both the head and body of the flies. Thus, incor-

porating curcumin into the diet and subsequently decreasing AChE activity could potentially result in elevated levels of acetylcholine in the synaptic cleft. As a result, this increase in acetylcholine levels may enhance the efficiency of cholinergic neurotransmission in the flies. This finding aligns with a study conducted by Akinyemi et al. (2018), which similarly demonstrated that curcumin possesses the capacity to regulate AChE activity and improve the antioxidant status in *D. melanogaster*. These findings align with previous findings in rodents, as reported in studies conducted by Shen et al. (2013) and Mythri and Srinivas Bharath (2012). For a considerable time, agents that possess anti-monoamine oxidase, as well as substances that have anticholinesterase properties, have been suggested as potential therapeutic options to alleviate symptoms associated with AD and PD, particularly memory loss and impaired motor coordination (Jellinger, 2009). Therefore, the decrease in the AChE and MAO activities noticed in *D. melanogaster* exposed to Hg toxicity and fed with diets supplemented with curcumin, as observed in this study, provides additional evidence of their potential as anticholinesterase and anti-monoamine oxidase agents. These findings further support the neuroprotective properties associated with curcumin. Additionally, it is noteworthy that the decrease in MAO activity in *D. melanogaster* exposed to Hg toxicity and fed with diets supplemented with curcumin is accompanied by a diminishment in the levels of ROS in the corresponding *D. melanogaster* group. This is particularly intriguing considering that elevated MAO activity is commonly linked to increased production of ROS, as reactive species are secondary products of the oxidative processes catalyzed by MAO (Sturza *et al*., 2019). This observation is consistent with previous research where similar connections were found between a decrease in MAO activity and the liberation of ROS (Oyeleye *et al*., 2021; Oboh *et al*., 2022). The potential of curcumin to ameliorate neurodegenerative diseases is evident through its ability to inhibit MAO, preventing the oxidative deamination of monoamine neurotransmitters such as dopamine. This action helps to impede the progression of diseases characterised by such neurotransmitter alterations. Additionally, curcumin's MAO inhibition also contributes to ameliorating the concurrent generation of oxidative assaults that are typically associated with MAO enzymatic activity.

In this study, the response of AChE activities in the *D. melanogaster* to Hg exposure was observed to be dependent on does and tissue distribution. In the heads of the flies, 1 mM Hg elicited significantly reduced AChE activity, while 2 mM Hg significantly elevated it. However, in the body, both doses caused a significant reduction in the enzyme´s activity when compared to the control group. While the full mechanisms behind these varying observations might not be fully unravelled in this study, it provides unique information on mechanisms of Hg toxicity that deserves further study. For example, the ability of Hg to significantly reduce or increase AChE activity in the neural tissue of *D. melanogaster* might be unique to Hg-neurotoxicity and provides an opportunity to study AChE-

mediated neurotoxicity through both stimulation and inhibitory pathways. This is more so because excessive inhibition and stimulation of AChE have been associated with several neurotoxicological conditions and neurodegenerative diseases; Several neurotoxic agents, such as organophosphates, metals, and toxic natural agents, act as AChE inhibitors, while elevated AChE activity has been linked to several neurodegenerative diseases, such as AD and PD (Ademiluyi *et al*., 2016; Jokanović, 2018; Walczak-Nowicka and Herbet, 2021). However, in both situations, curcumin acts as a therapeutic agent, thus further supporting the neuroprotective properties of curcumin (Chin *et al*., 2013; Akinyemi *et al*., 2017; Ogunsuyi *et al*., 2023).

Conclusion

The current research highlights curcumin as a promising treatment option for mitigating the adverse effects of Hg toxicity. This was supported by its capacity to enhance survival rates, boost memory function, and improve locomotor performance in *D. melanogaster*. The underlying mechanisms responsible for the observed behavioral alterations may be linked to curcumin's antioxidant, anti-monoamine oxidase, and anticholinesterase effects. Consequently, this study provides valuable support for further clinical investigations focused on developing therapeutic options against Hg toxicity, such as the use of Hg-containing products like dental amalgam.

Conflict of Interest
None declared.

Authors' Contribution
O.B.O was involved in conceptualization, fund acquisition, supervision and proof-reading of the final draft of the manuscript; P.B.O was involved in data acquisition, data analysis and writing of the first draft of the manuscript; A.V.A was involved in data analysis and writing of the manuscript draft; G.O was involved in project administration, data curation and methodology

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