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ORIGINAL ARTICLE

Impact of Acute Lead Exposure on Cognitive Abilities in *Drosophila melanogaster* Larvae

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ABSTRACT
Metals are universal constituents of ecosystems and play a significant biologically essential function. Lead (Pb) is a toxic metal that is not required for any physiological function in the body, and it is a severe threat to the nervous system. Nevertheless, the processes through which Lead influences neurotoxicity and neuronal sensitivity, especially for senses, are not fully understood. The larvae of Drosophila melanogaster allowed us to examine how acute Lead induced disruption in olfactory response. This study therefore showed that Lead treatment led to a dose dependent reduction in olfactory sensitivity to ethyl acetate odor. More importantly, the response index of the larvae that were exposed to higher concentrations of Lead before was significantly lower than those of the control specimens that had not been exposed to Lead. These findings underscore the detrimental effects of Lead on the olfactory circuitry, indicating potential disruptions in sensory processing pathways. By unraveling the details of the Lead-mediated deficits in olfaction in Drosophila, our study contributes to a better understanding of the neurotoxic effects of heavy metal exposure on sensory function and behavior across species. Lastly, this study underlines the significance of inquiring into how contaminants in the environment affect sensory processing to ensure that no adverse effects associated with heavy metals' pollution extend to humans and wildlife.
Keywords: *Behavior, Drosophila melanogaster, Ethyl acetate (EA), Lead (Pb), Olfaction, Toxicity.*

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INTRODUCTION

Heavy metals are inherent constituents of our ecosystem. They are chemical elements that possess metallic properties and are found in the d, f, and p blocks of the periodic table. While their presence is crucial for life, an excessive amount of them in our body can lead to severe and sometimes fatal health problems [21]. However, some heavy metals are considered to be nonessential for living entities and Lead is one such undesirable heavy metal used since prehistoric times by humans, causing worldwide adulteration [5-6]. Lead, along with other heavy metals, is introduced into the human body via dermal contact, inhalation of contaminated dust particles, and ingestion of Lead-contaminated food, water, and vegetation, all of which are products of anthropogenic activities that disperse heavy metals in the natural environment [16-18]. The risk of complications is especially pronounced among populations residing in polluted urban and industrial zones, where environmental Lead exposure continues to be a significant issue across several nations. Lead primarily affects the nervous system, and its toxicity appears to be most pronounced in the developing brain [3]. Since Lead (Pb) is not a vital element for living organisms, no specific transporter exists to carry it. It has been reported that metal ions frequently compete for specific metal transporters [7]. The neuropsychological functioning of individuals varies throughout their lifetimes due to Lead exposure. *Drosophila melanogaster*, a fruit fly, is a widely studied eukaryotic organism with significant contributions to biology and human diseases, with genomic studies suggesting that up to 75% of human genes are conserved [2]. Due to the flies being low maintenance and having a very short generation time, this is why they are highly used in laboratories and this reduces the demand. Flies can be bred, handled and their gene manipulated in large quantities [17]. Olfaction, or the sense of

smell, is an important part of *Drosophila* behavior that regulates many life-sustaining tasks like as foraging, mating, and avoiding predators and poisons [1]. Due to its remarkable similarity to the olfactory systems of mammals and a high degree of conservation of its olfactory system, *Drosophila* is an ideal model organism for investigating the neural mechanisms underlying olfactory perception and behavior [20]. *Drosophila* olfactory systems enable the identification of a vast array of volatile compounds, which are crucial for locating food, finding partners, and ovipositional sites. Although comparatively less complex than the olfactory system found in vertebrates, the *Drosophila* olfactory system adheres to the identical structural principles [8]. Due to its high toxicity and persistent accumulation, Lead has emerged as a highly hazardous metal to human health, particularly with concern to the nervous system [15]. Applying genetic tools, behavioral assays, and neuroanatomical techniques, the research on the influence of Lead on *Drosophila* olfaction provides valuable insights into the neurotoxic consequences of Lead on sensory processing and behavior in various species.

MATERIAL AND METHODS

Fly maintenance and breeding of the *Drosophila melanogaster* wild-type flies of the Oregon R+ strain was done in cornmeal medium, and they were kept at the 25 ^{0}C temperature in a BOD (Biochemical oxygen demand) incubator under a 12 h light/dark cycle. The medium was prepared with high-quality Agar in the concentrations of 8 gm/l, 15 gm/l Yeast extract, 80 gm/l corn, 20 gm/1 Glucose, and Sucrose 40 gm/l. One milliliter of the antifungal agent and antibacterial agent 0.125 Orthophosphoric acid dissolved in the medium (4 ml/l Propionic acid and 0.6 ml/l Orthophosphoric acid, respectively) was kept. The chemicals were procured from HiMedia (Mumbai, India).

Chemicals

The larvae were treated with Lead acetate ($Pb(C_2H_3O_2)_2$) with a purity of 90% (molecular weight 379.23 g/mol). Various concentrations of Lead acetate solution were prepared using a 5% sucrose solution as the solvent. The compounds employed for larval isolation were Monopotassium phosphate (KH_2PO_4), Calcium chloride ($CaCl_2$), Sodium chloride (NaCl), Potassium chloride (KCl), Disodium hydrogen phosphate (Na_2HPO_4), and Polyethylene glycol–6000 (PEG 6000). The previously mentioned tests utilized high-quality chemicals sourced from HiMedia. The ethyl acetate (EA) odorant of the highest grade and the mineral oil diluent were acquired from Sigma-Aldrich. The treatment and behavioral studies were carried out in glass Petri dishes with a diameter of 90 mm that were acquired from Borosil, a company based in India. The larvae were handled with Faber Castell paint brushes with delicate and supple bristles. In order to separate the larvae, strainers with a fine screen were acquired at the nearby market.

Larvae isolation and Lead treatment

An average of 150–200 fruit flies were sweeping and moved to fresh corn meal media bottles before being placed in BOD incubator (25 ^{0}C) for egg-laying. After laying the eggs for twenty hours, the flies were transferred to another corn meal media bottle, with no more fly left in the bottle with laid eggs. The bottles with eggs were then kept at an optimum temperature of 25 ^{0}C for a period of three days before further development in the BOD incubator. The development of early third instar larvae from eggs took approximately 72 hours (three days). In neurobehavioral investigations employing behavioral assays, larvae in their early third instar stage were utilized. The larvae were extracted by carefully accumulating the uppermost layer of corn meal media in a strainer while using a soft paintbrush, ensuring that no harm was inflicted on the larvae. To separate larvae from media, coarse media was transferred into a vial containing 30% PEG-6000 solution. Media debris sank to the bottom, while larvae floated to the top. The top layer was rinsed under gently running water at optimum temperature, and larvae were collected into a Petri plate with Ringer's solution, maintaining osmotic balance. 128 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 0.9 mM Na_2HPO_4, and 0.37 mM KH_2PO_4 were the components of the Ringer's solution [14]. Lead was administered to larvae at specific concentrations of 20 mM and 25 mM after their collection from media bottles as third-instar larvae. A thin layer of agar was poured on a Petri plate through the inoculation of 20 mL of a 1 percent Agar solution. In order to treat the larvae, 2.5 mL of Lead acetate solutions at the specified concentrations were poured onto the agar layer. In comparison to the control containers, treatment with Lead acetate resulted in an average 50 percent reduction in fly survivability at 25 mM concentrations over a 24-hour period [10-12]. The Lead acetate lethal concentration (LC) was calculated using Probit regression analysis. Based on the Probit analysis, these concentrations were chosen to investigate the impact of Lead on *Drosophila* (unpublished data). The harvested larvae were treated for seventeen hours on an Agar Petri plate containing Lead acetate solution. Following olfactory evaluation, these treated larvae were trained and their memory formation was assessed.

Behavioral experiments Larval plate assay

A Petri dish was prepared with a thin layer of 1 percent agar solution, which had been prepared using Ringer's solution. Approximately 10 mL of this agar solution was poured into the dish, and two small circular filter discs were positioned diametrically opposite to each other, within two arcs located 20 mm from the edge of the dish (Figure 1). Each filter disc was treated with 20 µL of an ethyl acetate (EA) odorant, diluted in mineral oil to a concentration of 10^{-2}. Around 50 larvae were placed at the center of the Petri dish (S zone) immediately before the odorant was applied. The larvae initiated movement toward the odor source, and after 2 minutes, photographs were taken to assess the distribution of larvae across the defined zones. The response index (RI) was subsequently calculated, and manual counting of the larvae was performed for verification.

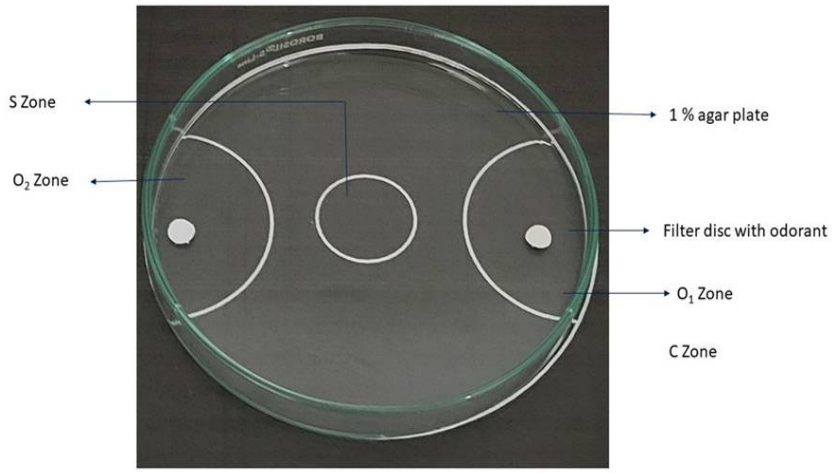

Figure 1: Larval plate assay

$$\frac{Number\ of\ larvae\ in\ zone\ 1\ (O1) + Number\ of\ larvae\ in\ zone\ 2\ (O2)}{Total\ number\ of\ larvae\ (O1 + O2 + C)}$$

Statistics

The statistical significance of differences between response indices (RI) of untreated and treated larvae were estimated by parametric tests of ANOVA. Similarly, the student's t-test analysed the significance of relative response of treated to untreated larvae.

RESULTS AND DISCUSSION

The investigation examined the impact of Lead (Pb) exposure on larval olfaction, particularly in response to ethyl acetate (EA) odor. It was observed that as the concentration of Lead treatment increased, the olfactory response in larvae gradually declined. The average response index (RI) of untreated larvae stood at 80.29 percent. However, exposure to Lead at concentrations of 20 mM and 25 mM decreased average olfactory response index to 68.85 percent and 56.39 percent, respectively. The disparity between the response of control larvae and those treated with 20 mM Lead for EA was 11.44 percent. In contrast, a 23.9 percent deviation was noted in the response of larvae treated with 25 mM Lead compared to the control group. This study highlighted the influence of Lead on the olfactory system of *Drosophila* larvae, particularly in their response to EA odor. The findings are graphically represented in Figure 2, the olfactory response of untreated larvae and those treated with Lead at 20 mM and 25 mM concentrations. Further analysis (Figure 3) revealed a relative decrease in response indices of treated larvae at 20 mM and 25 mM, compared to the control larvae, by 14.22 percent and 29.75 percent, respectively. The mean ± SEM of the 20 mM Lead-treated larvae compared to the control group was measured at 14.22 ± 1.89, while for the 25 mM Lead-treated larvae, it was 29.75 ± 1.77. Additionally, an average decrease of 15 percent in larval olfactory response was observed with the increase in Lead concentration from 20 mM to 25 mM.

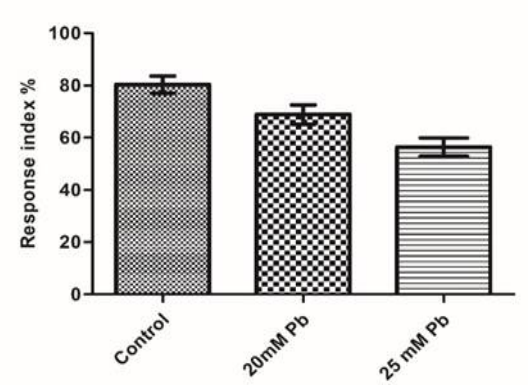

Figure 2: The average olfactory response index I (RI) of untreated (Control) and Lead-treated larvae (exposed to 20 mM and 25 mM concentrations) is represented. The decrease in larvae response to odor is evident from the bar graph with the error bar representing mean ± S.D. The One-way ANOVA analysis of the statistical significance of the difference in olfactory responses of larvae resulted in ($P < 0.0001$; R square = 0.90

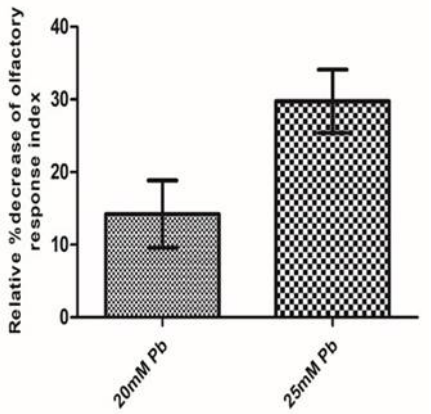

Figure 3: The bar graph represents the relative percentage decrease in the olfactory response (RI) of Lead-treated larvae at 20mM and 25mM concentrations with respect to untreated larvae (Control). Student t-test analysis was performed to determine the statistical significance of the mean olfactory response with the one-tailed P value < 0.0001.

The outcomes of this study give solid evidence of Lead exposure's adverse effects on olfactory behavior in *Drosophila melanogaster*. In a series of carefully planned studies, we revealed that Lead exposure affects numerous elements of olfactory perception and discrimination in fruit flies, offering information on the neurobehavioral effects of ambient heavy metal pollution. Neurological effects can emerge from both high-dose and low-dose exposures. Intellectual functioning is negatively correlated with blood Lead levels in children below 10 g/dL, according to recent research, with associations being stronger at lower exposure levels [9]. Our investigation revealed a significant correlation between exposure to Lead and cognitive impairment. Furthermore, we observed that Lead might have a direct impact on cognitive function. The exposure of Lead considerably impacted the olfactory response of early third-instar larvae. Furthermore, as the quantity of Lead was heightened, the olfactory response index (RI) diminished even more. The results of this study unequivocally demonstrate that Lead exposure has a significant impact on the brain circuit responsible for detecting odors. As a result, the reaction of larvae treated with Lead to the smell of ethyl acetate diminished compared to untreated larvae. This indicates that Lead exposure interferes with the sensory processing of olfactory cues, either by modifying the functioning of olfactory receptor neurons or the brain circuits responsible for detecting and distinguishing odors [19]. The causes of Lead induced decline in smell in *Drosophila* are likely complex and can involve changes in several molecular and cellular processes. Lead has been found to interfere with calcium signalling, oxidative stress pathways, and neurotransmitter systems in the nervous system. These effects may be responsible for the reported impairments in olfactory behavior [4-11-13]. By revealing the pathways by which Lead

affects olfactory behavior in *Drosophila*, our research adds to a better understanding of the neurotoxic impacts of heavy metal exposure on sensory function and behavior across species.

CONCLUSION

Our results show that olfactory response in fruit fly larvae are adversely affected due to exposure of Lead in dose-dependent manner. Thus, our findings underscore the need for exploring effects of environmental toxicants including Lead on sensory function and behavior in model organisms including *Drosophila melanogaster*. Our study will help exploring the ways of understanding and alleviating the ill-effects of Lead on different systems of organisms, minimizing health risks related to heavy metal toxicity, in people, the environment and animals.

CONFLICTS OF INTEREST: None

ETHICS

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

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AUTHORS CONTRIBUTION

Shubham Gudadhe: Conceptualization, Writing - original draft, Methodology, Data curation, Software. Sushma Kumari Singh: Software. Jawaid Ahsan: Validation, Supervision.

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