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Effect of Acute Exposure of Lead on Locomotion in *Drosophila melanogaster* Larvae

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Abstract

Environmental contamination by heavy metals poses a significant threat to both ecological and human health. Lead (Pb), a highly toxic heavy metal, is known to cause severe neurotoxic effects, particularly affecting cognitive and motor functions. This study investigates the impact of lead exposure on the locomotory behavior of *Drosophila melanogaster* larvae, serving as a model for understanding lead-induced neurobehavioral impairments. Third-instar larvae were exposed to 20 mM and 25 mM concentrations of lead acetate, and their locomotion was assessed using a crawling assay. Results indicated a significant decline in larval motility in a concentration-dependent manner. The impairment in movement suggests lead-induced disruption of neuronal signaling, synaptic plasticity, and oxidative stress. These findings align with previous studies on lead toxicity, which report neurodevelopmental deficits and motor dysfunctions in various animal models. Given the genetic and neurological similarities between *Drosophila* and mammals, this study provides valuable insight into the broader implications of lead exposure on nervous system function. Understanding these effects is crucial for developing strategies to mitigate lead toxicity and prevent its long-term consequences on neurobehavioral health.

Key words : Third instar larvae, *Drosophila melanogaster*, lead, locomotion, crawling.

Environmental pollution caused by various contaminants has become a major concern for ecological health and sustainability. Among these pollutants, heavy metals pose a significant threat due to their ability to disrupt ecosystems and negatively impact living organisms. One of the most toxic heavy metals is lead (Pb), which has been widely studied

for its harmful effects on both human and animal health.[1,6]

Lead (Pb) is a hazardous heavy metal with widespread environmental presence, posing significant public health concerns globally. It originates from various sources, including contaminated drinking water, lead-acid batteries, gasoline, old paint, canned food, traditional folk medicines, plumbing materials, lead-glazed ceramics, crystal glassware, cosmetics, jewellery, cigarette smoke, and even certain toys and vinyl lunchboxes produced using lead-based solder. Due to its persistence in the environment, ability to spread, and multiple exposure pathways, lead contamination remains a critical issue, necessitating continuous monitoring and mitigation efforts to safeguard human health.[11]

Recent research has demonstrated that lead (Pb) exerts toxic effects on multiple organ systems, including the nervous, hemato-poietic, skeletal, digestive, cardiovascular, urinary, reproductive, and immune systems. Among these, the neurological damage is particularly severe and of critical concern. Exposure to lead has been linked to impaired cognitive development in infants, reduced intellectual capacity, and diminished academic performance in adolescents. Furthermore, maternal lead exposure during pregnancy and lactation has been associated with negative impacts on the mental health of offspring, highlighting the long-term consequences of prenatal and early-life exposure.[17]

Lead is a hazardous element that contaminates air, soil, and water, often as a result of industrial and anthropogenic activities. Exposure to lead has been linked to a range of serious health problems, including blood disorders and neurotoxicity. While heavy metals are naturally occurring elements essential for certain biological processes, their excessive accumulation in the body can lead to severe and potentially life-threatening conditions.[2,15]

Lead interferes with numerous physiological and biochemical functions, including metabolism and organ health, contributing to disease development. Medical intervention becomes necessary when blood lead levels reach approximately 0.23 mg/L (10 µg/dL). One of the primary sources of lead accumulation in living organisms is contaminated food, which has prompted regulatory bodies like the Centers for Disease Control and Prevention (CDC) to establish safety guidelines for permissible exposure levels.[9]

Drosophila larvae provide a powerful genetic model for studying behavioral responses to various sensory stimuli and the underlying neural mechanisms. The neuronal circuitry responsible for larval peristalsis includes different types of neurons, such as motor neurons, interneurons, and sensory neurons.[7] This model offers valuable insights into the neurological effects of lead exposure, shedding light on how this heavy metal influences nervous system function and behaviour.

Fly Care and Husbandry :

Wild-type *Drosophila melanogaster* of the Oregon R+ strain were maintained on a cornmeal based diet under controlled laboratory conditions. The flies were kept at a constant temperature of 25°C with a 12-hour light/dark cycle in a BOD incubator. Their diet

consisted of high-quality polenta (corn), glucose, sugar, agar, and yeast powder, supplemented with antifungal and antibacterial agents, including propionic acid and orthophosphoric acid, sourced from HiMedia (Mumbai, India). To ensure optimal breeding, development, and overall health, the flies were routinely transferred to fresh culture bottles containing fresh media.

Chemicals :

Lead acetate (molecular weight: 379.33) was used for larval treatment, with varying concentrations prepared in a 5% sucrose solution as a solvent. For larval isolation, polyethylene glycol-6000 (PEG 6000), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), disodium phosphate ($Na_2 HPO_4$), and monopotassium phosphate (KH_2PO_4) were utilized. All chemicals were of high purity and sourced from HiMedia. Ethyl acetate (EA), used as a reinforcing odorant, was of high-grade quality and procured from Sigma-Aldrich.

Larvae isolation and Lead acetate treatment:

Approximately 150–200 randomly selected *Drosophila melanogaster* adults were transferred into fresh media bottles and placed in a 25°C incubator to facilitate mating and egg-laying. After 20 hours, the adult flies were removed, and the bottles were kept under controlled conditions for an additional three days to allow the eggs to develop. By 72 hours, third-instar larvae had fully developed and actively burrowed into the cornmeal media. These larvae were collected for neurobehavioral analysis.

To isolate the larvae, the upper layer of the cornmeal medium was gently removed using a fine strainer and a soft-bristled paintbrush to prevent any damage. The larvae, along with the coarse media, were transferred into a vial containing a 30% polyethylene glycol-6000 (PEG-6000) solution (prepared by dissolving 300 g of PEG-6000 in 1000 mL of distilled water). Due to differences in relative density, the larvae floated to the top while the media settled at the bottom. The floating larvae were carefully poured into a strainer and rinsed twice with distilled water to remove any residual PEG solution. The cleaned larvae were then transferred to a Petri dish containing 0.5 mL of Ringer's solution to maintain osmotic balance and prevent desiccation until further experimental procedures. The Ringer's solution was prepared by mixing two different solutions (Solution A and Solution B) in a 1:1 ratio, comprising 128 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 0.9 mM Na_2HPO_2, and 0.37 mM KH_2PO_4.

Following larval isolation, the third-instar larvae were exposed to specific concentrations of lead acetate. For treatment, 20 mL of 1% agar solution was poured into a Petri dish, and 2.5 mL of lead acetate solution was added.

Using a probit regression model, the study estimated the LC_{50}, which is the concentration of inorganic lead at which half of the *Drosophila* population would die within 24 hour period. The study's x-axis and y-axis represent logarithmically scaled doses of lead supplied for fly treatment and the related probability of fly mortality, as determined using probit analysis.[5,14] It was essential to identify

a toxic concentration of lead to assess the acute impact of Lead on the brain and to study neurobehavioral changes. In order to do this, experiments were carried out with two distinct lead acetate concentrations: 20 mM and 25 mM.

The isolated larvae were placed in the agar medium containing lead acetate for a duration of 4 hours. After treatment, the larvae were used for various experimental assays to assess the impact of lead exposure.

Statistics :

All statistical analyses were conducted using GraphPad Prism software (Version 8.0.2, GraphPad Software, San Diego, CA, USA). To assess the significance of differences among the various treatment groups, a one-way ANOVA test was performed. Statistical significance was indicated by p-values, with *** ($p \leq 0.0001$) denoting highly significant differences.

Behavioral experiments :

For the larval crawling assay, a Petri plate was prepared by pouring 20 mL of a 1% agar solution, creating a thin agar layer to facilitate larval movement. The plate was then placed over a standard graph paper for measurement. Both treated and untreated larvae were tested for their ability to move. A larva exposed to a specific concentration of lead was carefully placed at the center of a marked square on the graph-lined Petri plate (Fig. 1). After allowing a brief resting period of three seconds, its movement was recorded for 15 seconds. The number of smaller grid lines (each measuring 0.5 cm) crossed by the larva within this time frame was counted, and the total distance travelled in centimeters was calculated accordingly.

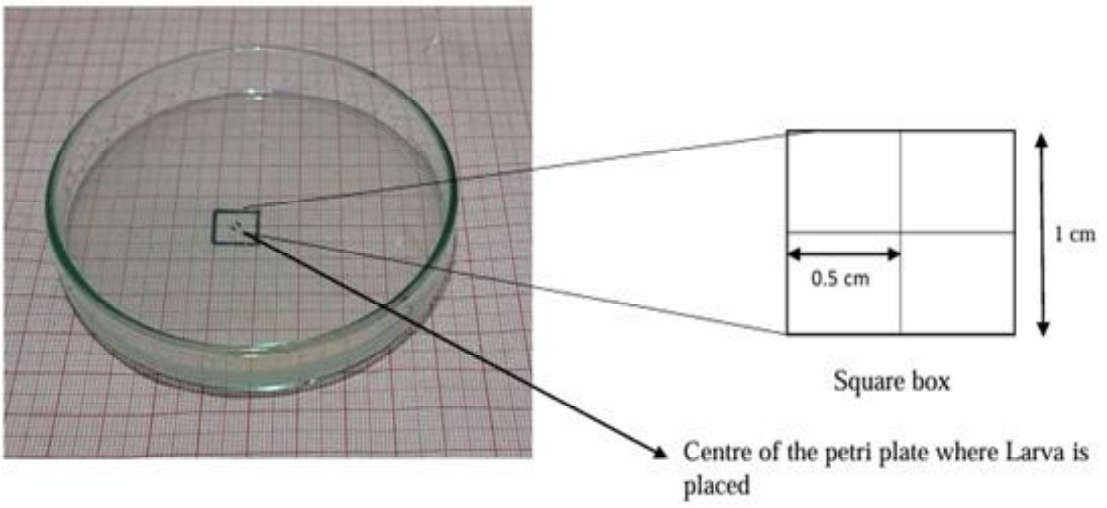

Fig. 1 Schematic representation of larval crawling assay. The number of smaller lines crawled by third instar larvae in 15 seconds are estimated to determine larva motility.

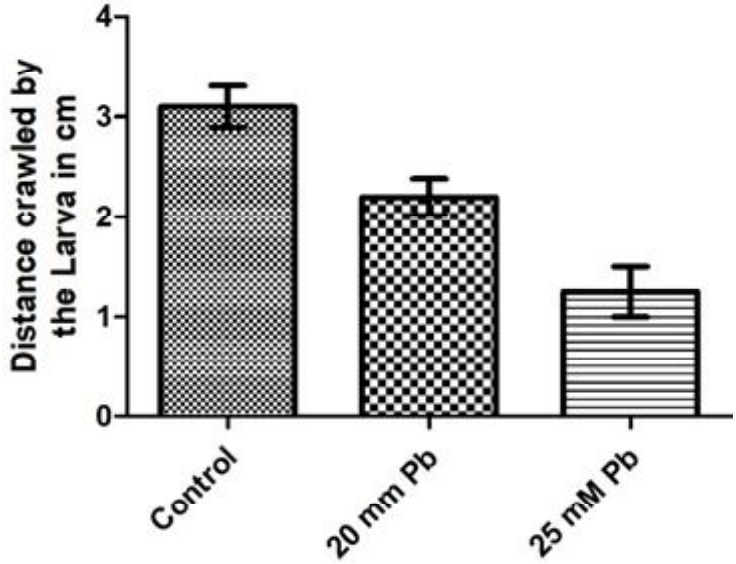

Fig. 2 The bar graph represents the crawling ability of untreated and treated (20 mM Pb and 25 mM Pb) third instar larva. Error bar represents mean ± S.D. One-way ANOVA analyzed the statistically significant difference (*p =0.001) between the different sample mean with 95% confidence interval and R squared value = 0.57.

More than 80 percent of the untreated larvae were able to crawl an average distance larvae were able to crawl an average distance of 3.1 cm within 15 sec. 20 mM and 25 mM lead treated larvae crawled an average distance of 2.2 cm and 1.25 cm within the duration of 15 sec (Fig. 2).

The locomotory activity of larvae significantly declined following exposure to lead. Larvae treated with 20 mM crawled a maximum distance of 3 cm and a minimum of 2.5 cm, while those exposed to 25 mM covered a range between 0.5 cm and 2.5 cm.

A noticeable reduction of 43.54% in crawling ability was observed in larvae treated with 20 mM lead compared to the untreated group. As lead concentration increased, the larvae's movement progressively decreased in a time-dependent manner. Specifically, larvae exposed to 25 mM lead showed a 62.36% reduction in crawling ability relative to the control group within the 15-second observation period. (Fig. 3).

Although the difference in movement between the 20 mM and 25 mM lead-treated larvae appears small, the data is statistically significant. The bar graph illustrates the average distance travelled by the larvae within 15 seconds. Higher concentrations of lead had a severe impact on larval movement due to its toxic effects. lead exposure has been shown to affect gene expression in a specific region of the *Drosophila* genome (~122 genes) involved in lead-induced changes in adult locomotion and to affect regulation of

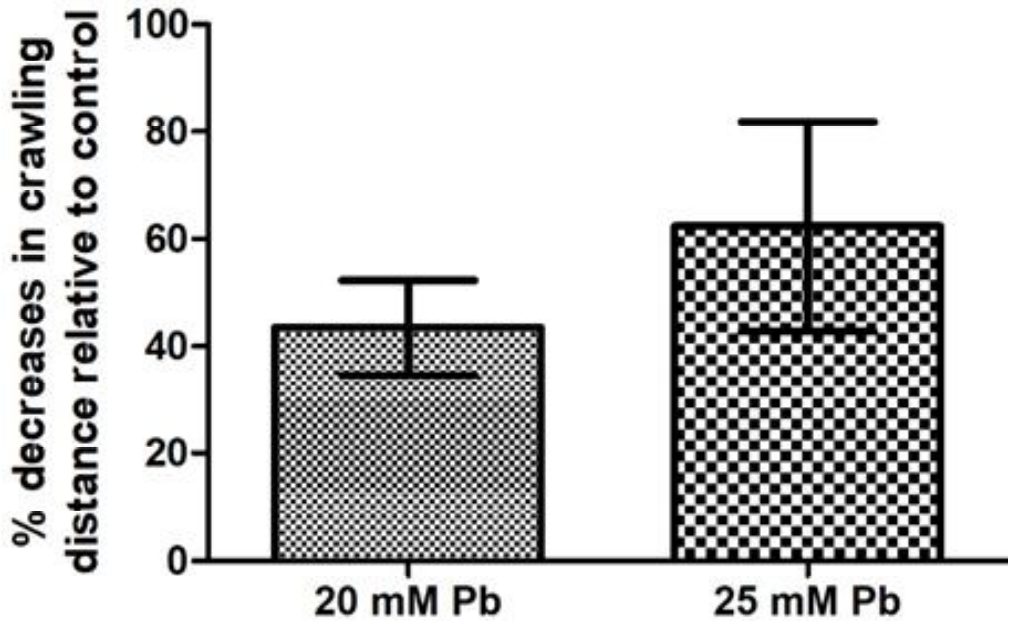

Fig. 3 The bar graph represents the relative percentage decrease in crawling activity of Lead treated third instar larvae at different concentrations with respect to untreated larvae. Student t- test analysed the significant difference between the Lead treated larvae motility at different concentrations (***P>0.0001)

intracellular calcium levels associated with neuronal activity lead exposure can block Ca2+ channels. In fact, exposure to heavy lead can also reduce *Drosophila's* cognitive skills and affect its sensory function.[8]

Previous studies have reported that heavy metal exposure disrupts neurotransmission, impairs synaptic plasticity, and leads to oxidative stress, all of which can contribute to reduced motor function.[12,13]

Additionally, the observed locomotion impairment aligns with previous research indicating that heavy metal toxicity in *Drosophila* serves as a model for understanding neurodegenerative disorders.[3] lead exposure has been linked to increased oxidative stress and mitochondrial dysfunction, both of which contribute to neuronal damage and behavioral deficits. Since *Drosophila* shares conserved neurological pathways with mammals, these findings provide valuable insight into the broader implications of lead toxicity on neuro-developmental and motor impairments.[10,16] In the present study, the crawling distance travelled by untreated third instar larvae was higher than the lead treated fruit fly larvae. It clearly indicates that exposure to inorganic lead led to the larval locomotory impairment. As the concentrations of lead was increased the crawling ability of larvae decreased significantly. The findings of present study corroborate with the previous study on rodent,

exposure to lead either delayed or prevented muscle formation affecting their locomotion.[4]

Both the 20 mM and 25 mM lead-treated *Drosophila* larvae demonstrated considerably reduced motility in a concentration-dependent manner when compared to the untreated control group, a symptom of neurological disease. Studies using rat models have shown that exposure to lead affects a number of behaviors and systems connected to aspects of memory, learning, motor function, and locomotion. Similar findings were obtained in this study of *Drosophila* larvae locomotory ability post-lead exposure providing an insight into the behavioral impairment due to lead.

In conclusion, our study highlights the detrimental impact of lead on larval locomotion in *Drosophila*, reinforcing the neurotoxic properties of this heavy metal. These findings contribute to a growing body of evidence on the harmful effects of environmental pollutants on neurobehavioral functions and underscore the importance of monitoring lead exposure in both ecological and biomedical contexts.

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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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