Evaluation of Peanut Skin Ethanol Extract as Protective Agent against Lead Acetate-Induced Reproductive and Neuromuscular Toxicities in *Drosophila melanogaster*

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Abstract

Lead acetate exposure is known to cause reproductive and locomotor toxicities in various organisms, including *Drosophila melanogaster*. This study aimed to assess the ameliorative effects of peanut skin ethanol extract (PSE) against lead acetate-induced toxicities in *Drosophila melanogaster*. Fly body weight, larval locomotor activity, fecundity, and eclosion activity were evaluated as endpoints. Lead acetate exposure reduced fly body weight, larval locomotor activity and fecundity, and hindered the eclosion of adult flies. Co-treatment with PSE at different concentrations (LP1, LP2, LP3) further exacerbated these toxic effects, except when co-administered with resveratrol (LR), which mitigated the lead-induced locomotor dysfunction and increased fecundity. Co-treatment with PSE (LP1, LP3) or PSE alone (P1, P2, P3) exceptionally mitigated lead induced weight loss and increased the fly body weight. Treatment with PSE alone showed a reduction in larval locomotor activity and did not significantly affect fecundity or eclosion. The observed effects may be attributed to the fatty acid content of the peanut skin extract, sensitivity of neuronal pathways, and potential interactions with lead. These findings suggest that peanut skin ethanol extract may have limited protective effects against lead-induced toxicities in *Drosophila melanogaster*, and further investigations are warranted to understand the underlying mechanisms and potential implications for human health.

Keywords: Lead acetate, peanut skin ethanol extract, *Drosophila melanogaster*, reproductive toxicity, locomotor toxicity, ameliorative effects.

INTRODUCTION

Peanut (*Arachis hypogaea*) is a tropical legume known for its rich nutrient composition and potential health benefits. While the focus of research has primarily been on the edible seed, peanut skin, which is often discarded, has gained attention due to its high content of bioactive compounds. Peanut skin is particularly notable for its abundance of metal chelators such as zinc and calcium, proteins, and antioxidants (Nepote *et al.*, 2020; Christman *et al.*, 2019). Moreover, it possesses one of the highest available levels of resveratrol, a polyphenol with recognized antioxidant, anti-inflammatory, and anticarcinogenic properties (Toomer, 2017). Considering these characteristics, exploring the potential benefits of peanut skin extract in counteracting the toxic effects of lead acetate becomes relevant.

Lead (Pb) is a hazardous heavy metal found in various environmental sources, including contaminated air, soil, and water. Exposure to lead has been associated with detrimental effects on human health, ranging from hematologic disorders to neurotoxicity (Borja-Aburto *et al.*, 1999; Safee *et al.*, 2014). The reproductive system is one of the targets of lead toxicity, with adverse effects observed on both male and female reproductive functions. Additionally, lead's impact on neuromuscular function has been documented, leading to locomotor impairments and neuropsychological disturbances (Safee *et al.*, 2014). Understanding the ameliorative potential of natural compounds against lead-induced toxicities is crucial for developing preventive strategies and protecting individuals from the deleterious effects of lead exposure.

Neuromuscular function plays a critical role in motor coordination, locomotion, and overall physical performance. The proper functioning of the neuromuscular system relies on the complex interplay between nerve cells (neurons) and muscle cells. Disruptions to this delicate balance, such as exposure to toxic agents, can result in impaired neuromuscular function. Lead acetate is known to interfere with neuromuscular signaling pathways, leading to locomotor dysfunction and motor deficits (Chen *et al.*, 2019). Investigating the potential ameliorative effects of peanut skin extract on lead-induced neuromuscular toxicity in *Drosophila melanogaster* provides a valuable model to study the underlying mechanisms and explore the protective properties of natural compounds.

Oogenesis, the process of egg cell development, is a fundamental aspect of female reproductive biology. It involves a series of tightly regulated cellular and molecular events that culminate in the production of mature oocytes capable of being fertilized. Any disruption to this delicate process can impair fertility and overall fecundity. Lead acetate exposure has been linked to adverse effects on oogenesis, including reduced egg laying rates and compromised reproductive success (Safee *et al.*, 2015). Understanding the impact of lead acetate on oogenesis and the potential ameliorative effects of peanut skin extract can provide valuable insights into the reproductive toxicity of lead and potential strategies for mitigating its harmful effects on fecundity.

The primary objective of this study is to evaluate the ameliorative effects of peanut skin ethanol extract against lead acetate-induced reproductive and locomotor toxicities in *Drosophila melanogaster*. We aimed to assess the effects of peanut skin ethanol extract on egg laying rate and oogenesis and locomotor activity in lead-exposed Drosophila.

MATERIALS AND METHODS

Chemicals and Peanut Skin Extraction:

Resveratrol (Candlewood Stars Incorporated, Danbury, USA), lead acetate (Fisher Scientific Company, USA), Non-crushed peanut skin obtained from a local industry in Keffi Town, Nasarawa State, Nigeria, was extracted by maceration with 70% ethanol at a ratio of 20 ml/g.

Experimental Design:

i. Group N: Normal control
ii. Group L: PbAc 0.4mM
iii. Group LP1: PbAc 0.4mM + PSE 7.2mg
iv. Group LP2: PbAc 0.4mM + PSE 14.4mg
v. Group LP3: PbAc 0.4mM + PSE 28.8mg
vi. Group LR: PbAc 0.4mM + RSV 6.2mM
vii. Group P1: PSE 7.2mg
viii. Group P2: PSE 14.4mg
ix. Group P3: PSE 28.8mg

Drosophila Diet Preparation (850ml)

850ml of distilled water was divided into 700ml and 150ml portions. The 700ml portion was added to a cooking pot and boiled. A small quantity of the boiling water was used to dissolve 10g of yeast. Then, 8g of agar-agar was added to the remaining boiling water and stirred for 10 minutes. The 150ml portion of water was used to dissolve 50g of cornmeal. The dissolved cornmeal was added to the boiling agar-agar mixture and stirred for another 10 minutes. The dissolved yeast was added to the simmering mixture and stirred periodically while allowing it to simmer for 15 minutes. The food was then cooled to 60°C, and 1g of Nipagin previously dissolved in 1ml of ethanol was added and stirred. After cooling to about 35°C (semi solid state), aliquots of the media were supplemented with the appropriate concentration of extract/drug and dispensed into vials (10-30cm) or as required. The remaining food was refrigerated until needed. Flies could be added as soon as the media solidified and cooled (Sani *et al.*, 2023).

Drosophila Stock Preparation and Culture

The study was conducted at the Drosophila and Neurogenetics Laboratory, Department of Zoology, Ahmadu Bello University, Zaria, Nigeria, under a 12-hour light and 12-hour dark cycle at 25°C. Age-matched wild Harwich strains of *Drosophila melanogaster* were obtained and separated into different sex groups, with male flies identified by their thick black band, rounded abdomen, and darker last two stripes on the abdomen. Female flies had a larger body size, approximately 25% larger, and a pointed abdomen. To facilitate the handling, the flies were temporarily immobilized (anaesthetized) by placing them on ice.

To establish a working stock culture, 30 male and 30 female adult flies were transferred to 8-ounce glass vessels containing normal growth medium. The parents were allowed to lay eggs for 3 days before being removed from the vials. The eggs underwent complete metamorphosis, and adults started to emerge from the 10th day, which were then used as the stock for the experiment.

Dosages and Drug Delivery

A standardized method for incorporating drugs into food for Drosophila was utilized with slight modification (Kruger & Denton, 2020). Concentrations of lead acetate and resveratrol were prepared per 10g of diet based on their molecular weights (228.25g/mol and 325.29g/mol,

respectively). The concentrations of peanut skin extract (PSE) at 7.2mg, 14.4mg, and 28.8mg, lead acetate at 0.4mM, and resveratrol at 6.2mM were adapted from a previous preliminary study conducted by Sani *et al.* (2023)

Post Treatment Assessment of Drosophila Weight

Thirty (30) mixed sex adult flies of 1-2days old were cultured on 30g of respective drug supplemented media for 5 days. The weights of 20 flies per group were taken at the end of the experiment.

Larval Crawling Assay

Larval collection: using a paint brush 15 male + 15 female of 3-4 days old flies were transferred into an 8 ounce bottle containing normal diet, the flies were allowed to lay eggs for 24hour then removed and only eggs were incubated for 4 days for collection of wandering 3rd instar larvae. 50ml 20% sucrose was gently poured into the culture bottle and allowed to stand for 20mins; this allowed the 3rd instar larvae to float on the sucrose solution in the bottle. Larvae were collected gently using a serological pipette (with the tip cut off) and rinsed twice in a mesh basket using distilled water.

Larval Treatment: about 15 larvae were transferred into a beaker of 5% sucrose only (Control N) or 5% sucrose + drug concentrations (L, LP1, LP2, LP3, or LR), and were allowed to feed for 15minutes.

Locomotor Assay: Using a paint brush, an individual larva was transported onto the center of a 15cm petri dish containing 2% agar (previously prepared and hardened) placed over a graph sheet of 0.2cm^2 grid. Number of gridlines crossed in one minute was counted as readout for the locomotor activity (Nichols *et al.*, 2012).

Eclosion Assay

An 8 ounce bottle of 15 male + 15 female flies were allowed to mate and lay eggs for 16hours. Freshly hatched, age matched 2nd instar larvae were collected from bulk fly stock cultures using 20% sucrose as described above in larval collection under Larval crawling assay. Using a paint brush the floating 2nd instar larva were carefully collected and transferred into individual vials

with desired drugs concentrations, larvae were allowed to rear and pupariate. The pupae were marked on the side of vial and were observed further until the pupa ecloses. Then all the flies that have eclosed were counted, whether dead or alive. For each diet 3 replicates were setup with 20 2nd instar larvae in each vial (Abdulazeez, 2020).

Female Fecundity Assay

For Fecundity around 50 adult male and female flies of 1-2 days old were transferred into vials containing 30g of respective drugs infused diets and were cultured for 7-15days. Everyday a new female is collected from the culture vials and transferred into an egg laying chamber for 16-18hours, using a dissection microscope eggs laid were counted (Abdulazeez, 2020).

RESULTS

Post Treatment Assessment of Drosophila Weight

Lead acetate reduced the adult Drosophila weight compared to the normal control group. Co-treatment with PSE at concentrations of 7.2mg (LP1) significantly ameliorated the toxicity of lead and significantly increased the weight of Drosophila. A moderate amelioration of weight loss was observed upon co-treatment with PSE 14.4mg (LP2) or RSV 6.2mM (LR). Co-treatment with PSE at a concentration of 7.2mg (LP2) did not have a significant effect on the fly weight. Treatment with PSE 7.2mg-28.8mg alone (P1, P2, or P3) considerably increased Drosophila weight (figure 1).

Larval Locomotor Activity

Lead acetate (PbAc) exposure significantly reduced larval locomotor activity, as indicated by a decrease in the number of gridlines crossed per minute compared to the normal control group. Co-treatment with peanut skin extract (PSE) at concentrations of 7.2mg-28.8mg (LP1, LP2, and LP3) further reduced larval locomotor activity. However, co-treatment with resveratrol (RSV) (LR) showed a considerable ameliorative effect on lead-induced locomotor dysfunction, allowing the larvae to cross more gridlines, even surpassing the normal control group. Notably, treatment with PSE at concentrations of 7.2mg-28.8mg alone also resulted in a reduction in the number of gridlines crossed and subsequently reduced larval locomotor activity (figure 2).

Fecundity Activity

Lead acetate or its co-administration with PSE at concentrations of 7.2mg, 14.4mg, and 28.8mg reduced the egg laying rate of *Drosophila melanogaster*. Only resveratrol at a concentration of 6.2mM (LR) ameliorated the toxicity and increased the fecundity of female flies. Supplementation of the Drosophila diet with PSE at concentrations of 7.2mg (P1), 14.4mg (P2), and 28.8mg (P3) did not significantly affect the fecundity of female flies compared to the normal control group; instead, it significantly reduced the egg laying rate (table 1).

Eclosion Activity

Lead acetate significantly reduced the larva to adult conversion rate compared to the normal control group. Co-treatment with PSE at concentrations of 7.2mg-14.4mg or RSV (LP1, LP2, and LR) exacerbated the toxicity of PbAc, further inhibiting the eclosion of adult flies. However, co-treatment with PSE at a concentration of 28.8mg (LP3) abolished lead toxicity to some extent by slightly increasing the eclosion rates of adult flies. Treatment with PSE at a concentration of 7.2mg alone did not have a significant effect on the fly eclosion rate (figure 3).

DISCUSSION

The treatment of Drosophila larvae with peanut skin extract was found to impair larval locomotor activity, reduce fecundity, and retard eclosion. The reduced larval locomotor activity may be attributed to the sensitivity of peristalsis-stimulating neurons, serotonin, and leucokinin to fatty acids, such as palmitic acid, which is a major component of the extract (Okusawa *et al.*, 2014; Moon *et al.*, 2014; Chen *et al.*, 2019). Furthermore, the non-ameliorative effect of peanut skin ethanol extract on PbAc-induced locomotor toxicity may be due to the high activation energy required to break down the fatty acid components of the extract for energy generation. Lipolysis, triggered by the activation of adenylcyclase, converts ATP into cAMP (Adamska-Patruno *et al.*, 2018).

Additionally, larvae are known to be active feeders during the developmental stages of Drosophila. The extract may have aided the assimilation of lead (Pb) since organic lead is highly lipid soluble and has the ability to interfere with Cu2+ activity, induce behavioral abnormalities,

and affect the developing nervous systems of organisms (Bhattacharjee *et al.*, 2018). Lead exposure has also been shown to increase the gestational period and impact fetal development.

The significant increase in weight observed in the flies could be attributed to the fatty acid content of the peanut skin extract. Obesity in the flies may be a contributing factor to the decline in egg laying rate, as obesity has been linked to male and female factor infertility in various studies (Chaudhuri *et al.*, 2022). Furthermore, the fatty acids linoleic and oleic acids have previously been reported to cause pathological effects such as gestational diabetes (Szczuko *et al.*, 2020), mitochondrial oxidative stress, and embryo underdevelopment (Yousif *et al.*, 2020).

CONCLUSION

It is worthy of note that this study found out that the peanut skin extract at the tested concentrations did not significantly ameliorate lead acetate induced toxicity on fecundity and locomotor but goes further to reduce the fecundity and locomotor functions. Even when PSE was administered singly to the flies, locomotor, fecundity and oogenesis were negatively impacted. Therefore the fatty acids detected by GC-MS analysis need to be characterized, isolated and individually tested for their modulatory effects on neuromuscular functions, fecundity and eclosion.

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Authors' Contributions:

Conceptualization: HS, BYM, TOB and ZMZ. **Methodology, Investigation and Formal Analysis:** HS and BYM. **Validation:** BYM, TOB and ZMZ. **Resources:** KTJ and JHL. **Original Draft Preparation:** HS. **Review and Editing:** BYM, TOB and ZMZ.

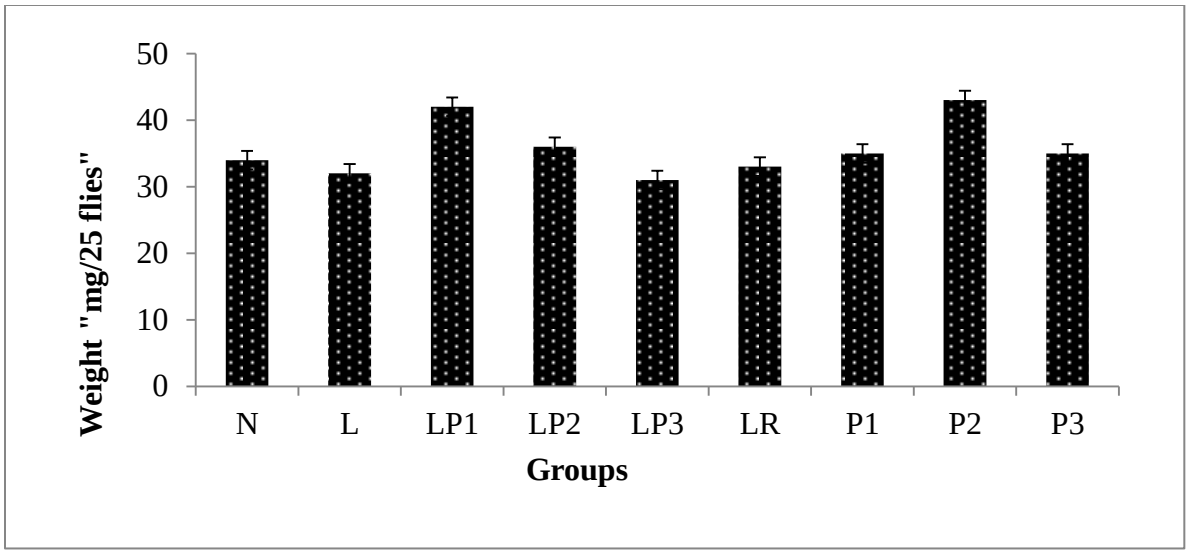

Figure 1: Post-treatment assessment of Drosophila body weight. Results are presented as Mean ± SEM**.**

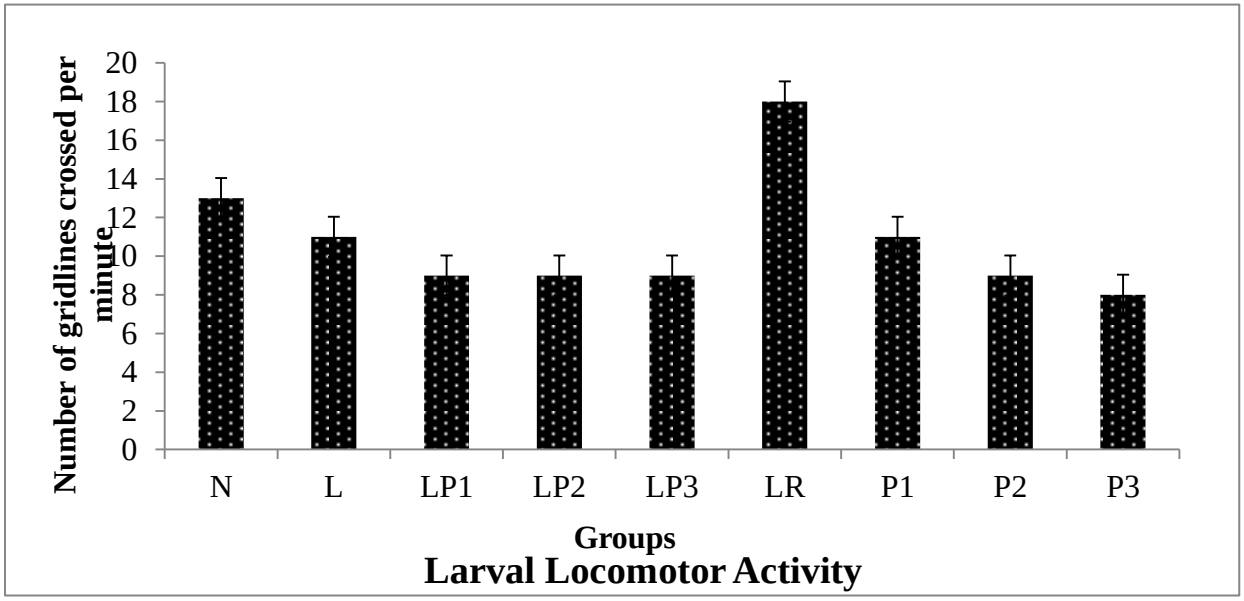

Figure 2: Larval locomotor activity showing the number of gridlines crossed by larvae in one minute. Results are presented as Mean ± SEM.

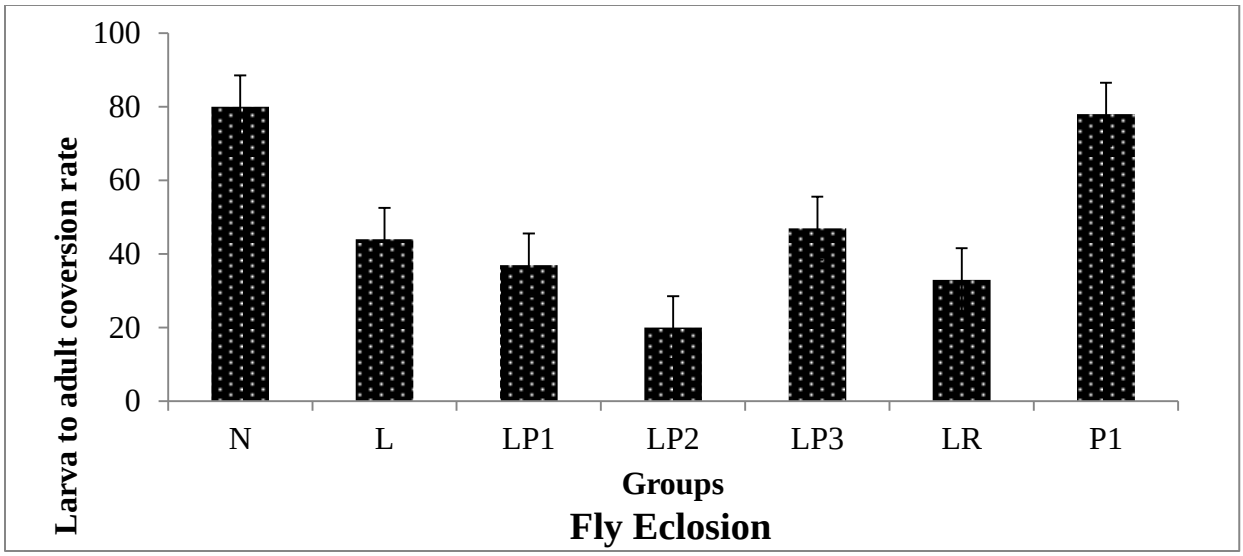

Figure 3: Drosophila eclosion activity showing the larvae to adults' conversion rates. Results are presented as Mean ± SEM.

Table 1: Fecundity activity showing the egg laying rates of female Drosophila.

Groups	**N**	**L**	**LP1**	**LP2**	**LP3**	**LR**	**P1**	**P2**	**P3**
Egg laying rates	70.3±2	23.7±4*	14±2*	15±1*	8±1*	31.3±1*	54±4*	59±15*	51±3*

Values with * denotes significant difference from the control (N) at $p<0.05$

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