THE MITIGATING EFFECTS OF CO- ADMINISTRATION OF RESVERATROL ON BIOLOGICAL AND BIOCHEMICAL PARAMETERS OF LEAD-POISONED ADULT *DROSOPHILA MELANOGASTER*

R. Abdulazeez[1*], S. M. Highab[2], U.F. Onyawole[1], M.T. Jeje[1], H. Musa[1], D. M. Shehu[1] and I. S. Ndams[1]

[1]Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]Department of Pharmacology and Therapeutic, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Federal University Dutse, Jigawa State, Nigeria.

*Corresponding author: rabdulazeez@abu.edu.ng/rashidat.int@gmail.com,; +2348185001701

Abstract
Lead toxicity is a major environmental issue and has been associated with various health problems such as cognitive and behavioral impairments, reproductive defects and cellular oxidative stress. The study investigated the potential of resveratrol in mitigating the effects of lead-induced toxicity in *Drosophila melanogaster*. Three (3) days old Harwich strain adult *D. melanogaster* were orally exposed to Pb (60 mg/L), Succimer (10 mg/kg), and Resveratrol (50, 100 and 150 mg/kg). Biological and biochemical parameters (ALP, AST, TB, CB, and electrolytes levels), kidney function test (Ur and Cr), oxidative stress marker (MDA), and antioxidants (SOD, and CAT) were evaluated. Resveratrol significantly increased egg-laying, eclosion rate, filial generation output, locomotor activity, and life span of *D. melanogaster* in a dose-dependent manner up to 150 mg/kg of diet. Most biochemical parameters investigated were significantly rescued in the lead-poisoned fruit flies ($p < 0.05$) when co-treated with resveratrol. The results suggest that resveratrol has a protective effect against lead toxicity in *Drosophila melanogaster* and could potentially be used as a therapeutic agent for lead poisoning in humans.

Key words: Fruit flies; Harwich strain; Lead toxicity; Resveratrol; reproductive capacity; Oxidative stress

Introduction

The presence of heavy metals poises significant environmental pollution and acts as a causative agent to myriads of diseases including kidney dysfunction (Tsai et al., 2017), nervous system disorder, cardiovascular diseases (Obeng-Gyasi *et al.*, 2018), gastrointestinal diseases (Balali-Mood *et al.*, 2021). Unlike organic matter, heavy metals do not decompose within a short time but rather accumulate in organs such as the kidney, liver, brain, and bone, which eventually leads to chronic poisoning and death (Rehman *et al.*, 2018; Cheng *et al.*, 2019). Lead (Pb) is a toxic heavy metal that is widely dispersed in the environment due to various human activities such as industrial emissions and the use of lead-based products. Lead toxicity is known to cause a wide range of negative effects on human health, including reproductive toxicity, neurological damage, and cardiotoxicity, with oxidative stress being the likely mechanism of action of this environmentally persistent toxicant (Flora et al., 2012; Rehman *et al.*, 2018; Cheng *et al.*, 2019). Lead in the environment enters human or animal bodies mostly via ingestion or inhalation.

Lead toxicity occurs both in children and adults, with a worldwide burden of childhood lead exposure estimated to cost 998 billion U.S dollars annually or 1.47% of the world's GDP in 2011 with lower- and middle-income countries (LMICs) bearing the brunt of the costs (IHME, 2017; Burki, 2020). The Institute of Health Measurement and Evaluation (IHME) estimates that due to the long-term adverse effects of lead exposure, 1.06 million people died, and 24.4 million people suffered morbidity in 2017, and lead induced idiopathic developmental intellectual disability accounted for 63.2% of the global burden in 2016, with low and middle-income countries bearing the heaviest burden (Li *et al.,* 2020). The presence of lead in gasoline, jewelry, traditional medications, toys, and foods have led to an increased worldwide environmental burden (Obeng-Gyasi, 2019). Given the growing concern over the negative health impacts of lead toxicity, it is crucial to identify potential strategies for ameliorating its toxic effects.

Resveratrol (3, 5, 4'-trihydroxystilbene) is a natural polyphenol commonly found in grapes, wine, peanuts, and soy (Pastor *et al.,* 2019). It is known to possess antioxidative, anti-carcinogenic, and anti-inflammatory (Burns *et al.,* 2002; Yeh *et al.,* 2013) and anti-aging properties (Baur *et al.,* 2006). Studies have shown that resveratrol can prevent DNA damage (Zhang et al., 2020). *Drosophila melanogaster* (vinegar fruit fly) is a significant invertebrate model in biomedical research due to 60% of the fruit fly genome is homologous to that of humans, easy handling and genetic manipulations, ideal for drug screening and serves as a bio-indicator (Bier, 2005; Adedara *et al.,* 2015). The use of the fruit fly is recommended by the European Centre for the Validation of Alternative Methods (ECVAM) for promoting the 3Rs (reduction, refinement, and replacement) of laboratory animal usage in toxicity studies, and testing (Benford *et al.,* 2000).

The mainstay of treating lead poison is the chelation therapy, which involves oral administration of succimer and penicillamine and parenteral administration of calcium disodium dimercaprol edaetate ($CaNa_2$ EDTA) (Kathura, 2020). The cost of using succimer; the conventional drug in the treatment of lead poisoning, is significantly expensive (Vahabzadeh, *et al.,* 2021), hence the need to search for readily available and cost-effective alternatives. Giving that Oxidative stress is the likely mechanism of action of lead poisoning (Obeng-Gyasi, 2018), and resveratrol is known to efficiently scavenge reactive oxygen species (Chandrashekara and Shakarad, 2011), in addition to being readily available and affordable even in very poor communities, this work hypothesized that resveratrol could be the potential cost-effective treatment for lead poison.

Materials and methods

Chemicals

All chemicals were of analytical grade. *Trans*-resveratrol (60g) was obtained from Candlewood Stars Incorporated, Danbury, USA (Batch Number: MR 110218), Succimer (Sigma-Aldrich, U.S.A), while lead acetate (LA) (product No; 10142, BDH Laboratory Chemicals Limited Poole, England).

Drosophila melanogaster stock and culture

D. melanogaster (Harwich strain) was obtained from Dr. A. Abolaji, University of Ibadan, reared in the *Drosophila* and Neurogenetics Lab., Department of Zoology, Ahmadu Bello University, Zaria on cornmeal containing 1% w/v agar, 1% w/v Brewer's yeast, 6% w/v corn flour and 0.1% nipagin. Flies were maintained at constant temperature and humidity (23 ± 2°C; 60% relative humidity, respectively) under 12 h dark/ light cycle conditions.

Evaluating biological parameters

Egg laying ability, filial generation output, and lifespan of resveratrol and lead acetate-treated flies were evaluated according to the procedures described by Chattopadhyay *et al.* (2015), Eclosion rate was assessed based on the method of Rand *et al.* (2014), and negative geotaxis assay as described by Ali *et al.* (2011).

Determination of biochemical parameters

To assess biochemical parameters, 50 flies (of both genders) were exposed to concentrations of lead acetate (60 mg/L), resveratrol (50, 100, and 150 mg/kg), and succimer (10 mg/kg) in 30 mg of diet for 28 days. At the end of the exposure, flies were anaesthetized using CO_2, weighed, homogenized in 0.1 M phosphate buffer, pH 7.0 (ratio of 1 mg:10 mL), and centrifuged at 4000 g for 10 min at 4°C in a Thermo Scientific Sorval Micro 17 R refrigerated centrifuge. Subsequently, the supernatants were separated from the pellets into labeled Eppendorf tubes, stored at -20°C. Liver function enzymes which included (aspartate transaminase (AST) and alkaline phosphatase (ALP) levels), total and conjugated bilirubin, serum electrolytes (calcium, chloride, sodium and potassium), kidney function parameters (urea and creatinine) were analyzed using automated biochemistry analyzer (Selectra XL, Vital Scientific, Netherlands) and kits obtained from ELITech reagent kits, Netherlands. Malondialdehyde activity was assayed according to the procedure of Niehaus and Samuelson, (1968). Catalase activity was determined using the method of Aebi, (1984). Superoxide dismutase activity was evaluated with the method of Fridovich, (1989). All the assays were carried out in triplicates.

Statistical analyses

The data were subjected to Levene's homogeneity of variance test and Shapiro-Wilk test for normality, before employing parametric statistics. One-way ANOVA was used to assess the significant differences among groups. Plots were made using the GGPLOT2package for R, and all statistical analyses were performed using R version 4.2.2 for macOS at a5% significance level.

Results

Effects of lead toxicity and resveratrol on selected biological parameters of D. melanogaster

Lead at 60 mg/L significantly reduced the number of eggs laid (fecundity), which was ameliorated by resveratrol in a dose-dependent manner, with flies reared on 150 mg/ kg resveratrol laying the highest number of eggs ($p < 0.05$) when compared with the control group. The standard drug for treating Pb poison (succimer) did not rescue egg laying ability of the lead poisoned fruit flies (Figure 1). The rate of eclosion (Figure 2) was significantly reduced in lead exposed flies. Resveratrol at 50 mg/kg significantly improved the rate of emergence in most of the studied generations.

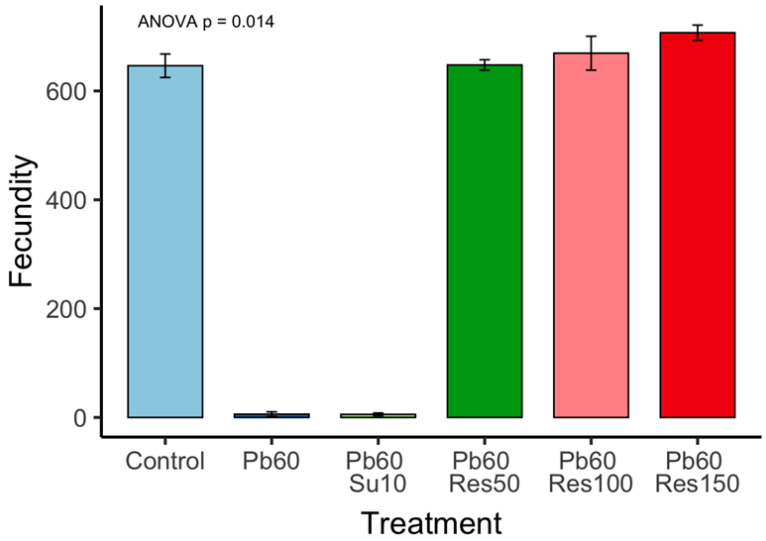

Fig. 1. Effects of Pb and resveratrol on egg-laying ability of Harwich strain *D. melanogaster*. Data are presented as Mean ± SEM per treatment group. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

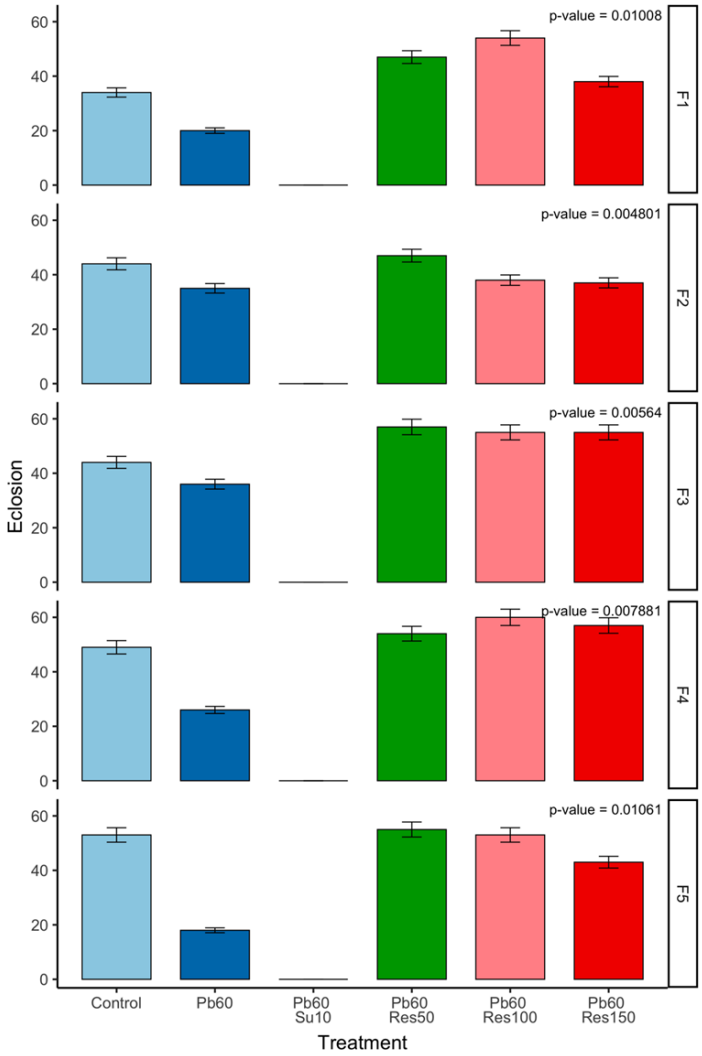

Fig. 2. Implications of Pb and resveratrol on eclosure rate of Harwich strain *D. melanogaster*. Data are presented as Mean ± SEM of 20 larvae/vial with 3 replicates per treatment group. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

Comparing the number of offspring produced across five (5) generations, flies exposed to only Pb showed significant transgenerational reduction in the filial generation output (Figure 3), which was significantly mitigated by resveratrol at 100 mg/kg in most generations. In addition, the locomotor activity for each filial generation (Figure 4) indicated a significant reduction in the climbing ability of flies exposed to Pb. The 150 mg/kg resveratrol treatment group significantly rescued the climbing abilities of the flies. The longevity of flies was significantly reduced by exposure to Pb (34 days; $p < 0.05$), which was significantly improved to 53 days when co-exposed to Pb and 100 mg/kg resveratrol (Figure 5; $p < 0.05$).

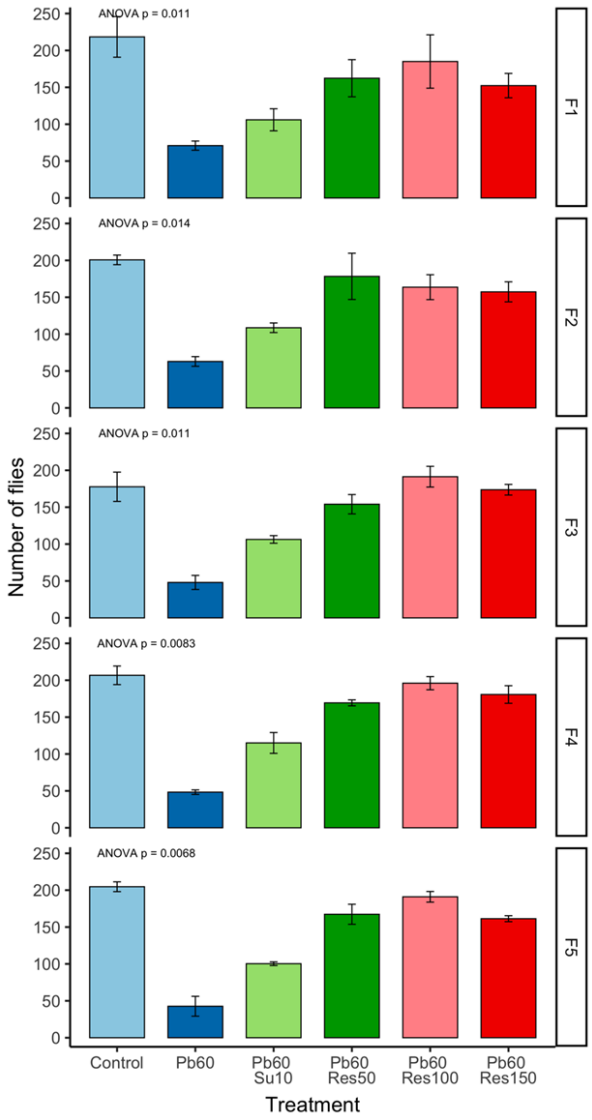

Fig. 3. Effects of Pb and resveratrol on filial generation output of Harwich strain *D. melanogaster*. Data are presented as Mean ± SEM number of emerged flies/vial with 3 replicates per treatment group. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

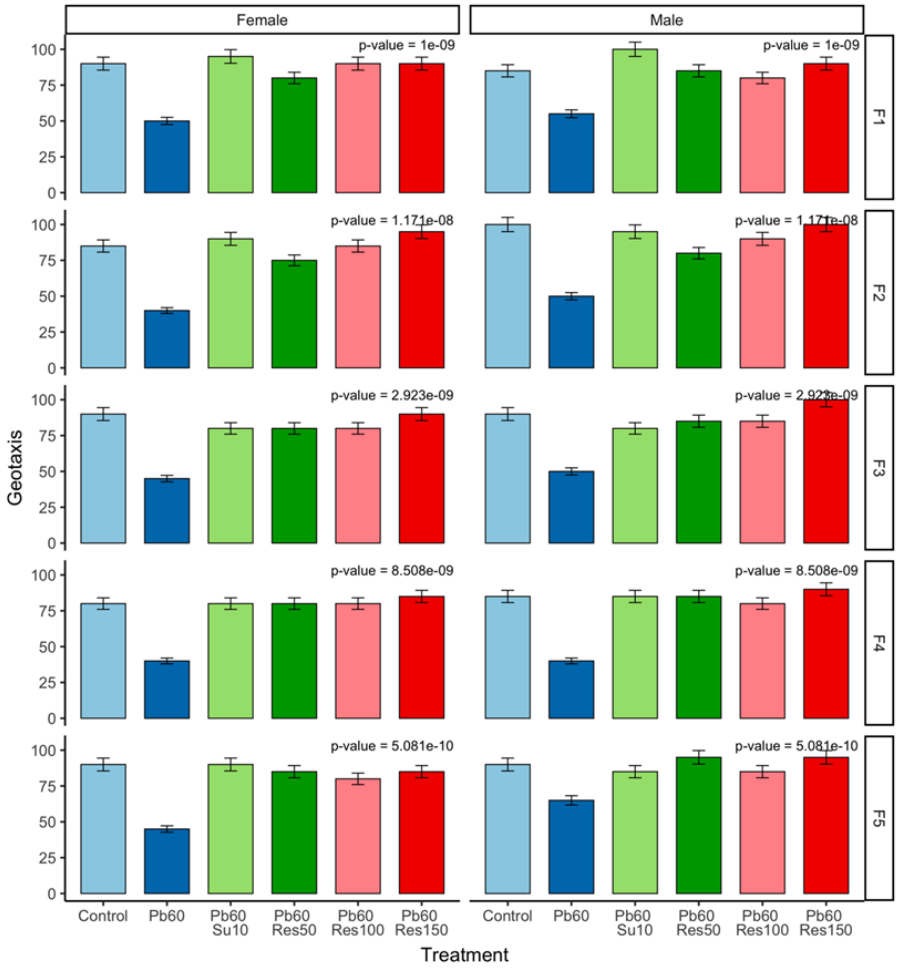

Fig. 4. Effects of Pb and resveratrol on climbing ability of male and female *D. melanogaster* Harwich strain. Data are expressed as percentage of flies to pass the 8 cm mark, and presented as Mean ± SEM of 10 flies per 10 trials for each treatment group/generation. Significance is $p < 0.05$. Pb: lead (mg/L), SU: succimer (mg/kg), RSV: resveratrol (mg/kg).

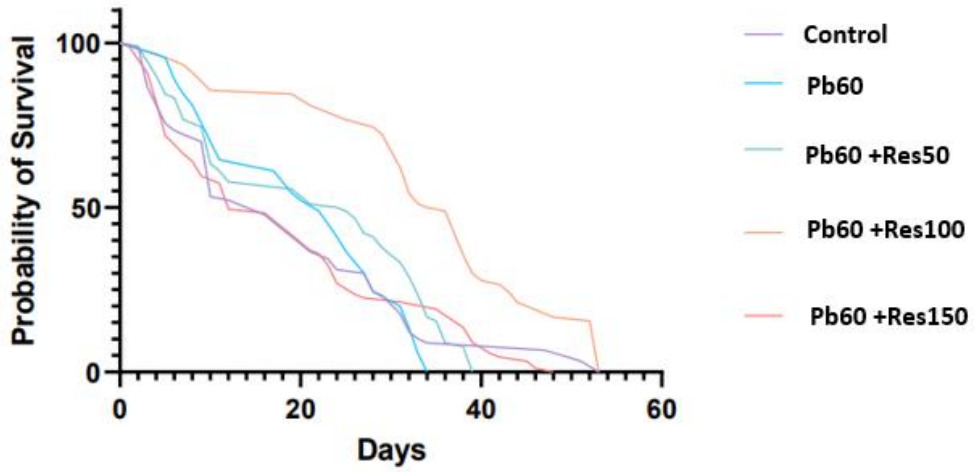

Fig. 5. Influence of Pb and resveratrol on lifespan of *D. melanogaster* Harwich strain. Data are presented as Mean of 50 flies/ vial in triplicate per treatment group. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

Roles of lead and resveratrol on selected biochemical parameters of lead poisoned D. melanogaster

Chronic exposure to Pb administered alone, or in combination with resveratrol resulted in changes in the investigated biochemical parameters, enzymes, co-enzyme, electrolytes, oxidative stress marker and antioxidants. There was significant increase in Ur, TB, and CB in the Pb exposed group when compared with the control ($p < 0.05$). The level of Ur was reduced in flies co-exposed to Pb 60 mg/L + Su 10 mg/kg, while TB and CB were reduced in flies co-exposed to Pb 60 mg/L + Res 150 mg/kg. The creatinine level was lower when exposed to Pb alone, and was significantly increased in the Pb 60 mg/L + Res 50 mg/kg group (Figure 5A-D).

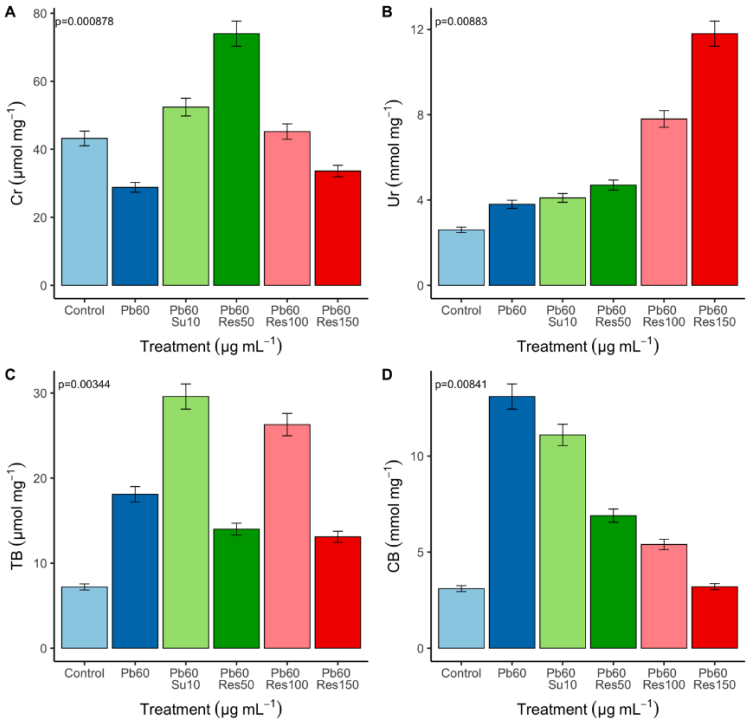

Fig. 5: Effect of lead and resveratrol on the kidney and liver functions of lead-exposed *D. melanogaster*. The data are presented as Mean ± SEM. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

Figure (6A and C) shows a significant increase in the levels of the enzyme AST and co-enzyme Zn in the group treated with Pb alone. However, in the Pb 60 mg/L + Res 150 mg/kg group, a significant decrease in the levels of both enzymes was observed. The group treated with Pb exhibited a significant decrease in the enzyme ALP compared to the control group. In contrast, the Pb 60 mg/L + Res 150 mg/kg group showed a significant increase in the ALP level (Figure 6B; $p < 0.05$).

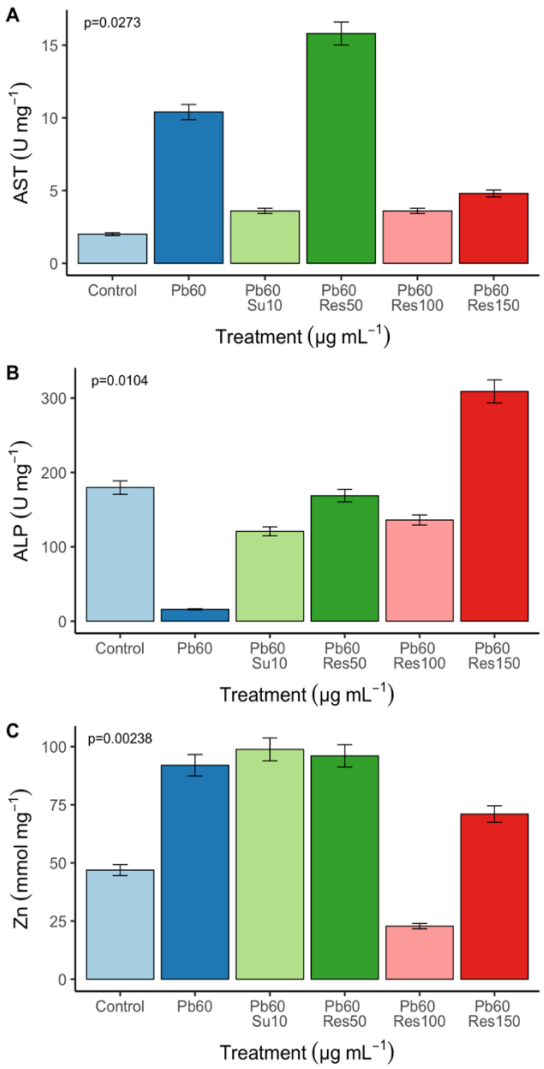

Fig. 6: Effect of lead and resveratrol on serum biochemical parameters of lead-exposed *D. melanogaster*. The data are presented as Mean ± SEM. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

Changes in the Ca and K electrolytes was towards reduction in the Pb treated group, but statistical significance was observed in the Pb 60 mg/L + Res 150 mg/g group for both electrolytes (Figure 7A and B). The Pb treated group exhibited an increase in the Cl and Na levels, with reductions in the Pb 60 mg/L + Res 150 mg/g group and Pb 60 mg/L + Res 100 mg/g group for Cl and Na, respectively (Figure 7C and D).

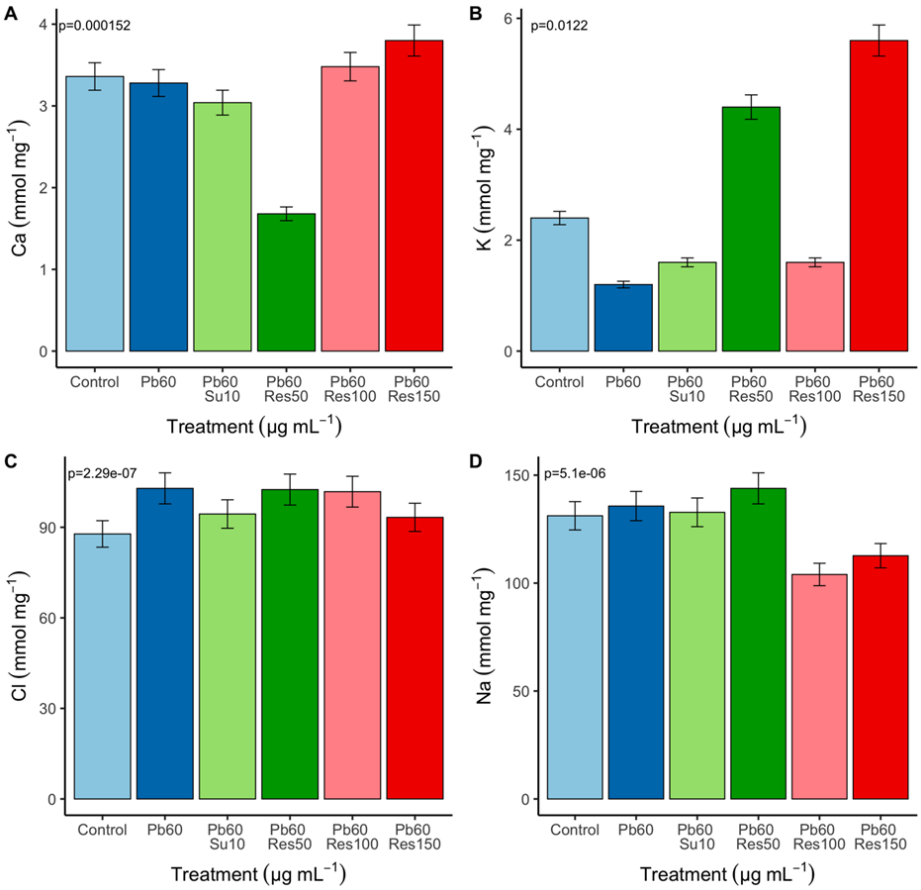

Fig. 7: Effect of lead and resveratrol on serum electrolytes of treatment groups. The data are presented as Mean ± SEM. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

All experimental group showed an increase in MDA and SOD levels, when compared to the control. However, a reduction ($p > 0.05$) in the oxidative stress marker and a significant increase ($p < 0.05$) in the antioxidant was observed in the Pb 60 mg/L + Res 100 mg/g group (Figure 8A and B). The activities of CAT in Pb and resveratrol-exposed flies were significantly different in all treated groups when compared with the normal control ($p < 0.05$). In addition, the Pb 60 mg/L + Res 50 mg/g group showed a significant increase in the CAT levels when compared to other treated groups (Figure 8C).

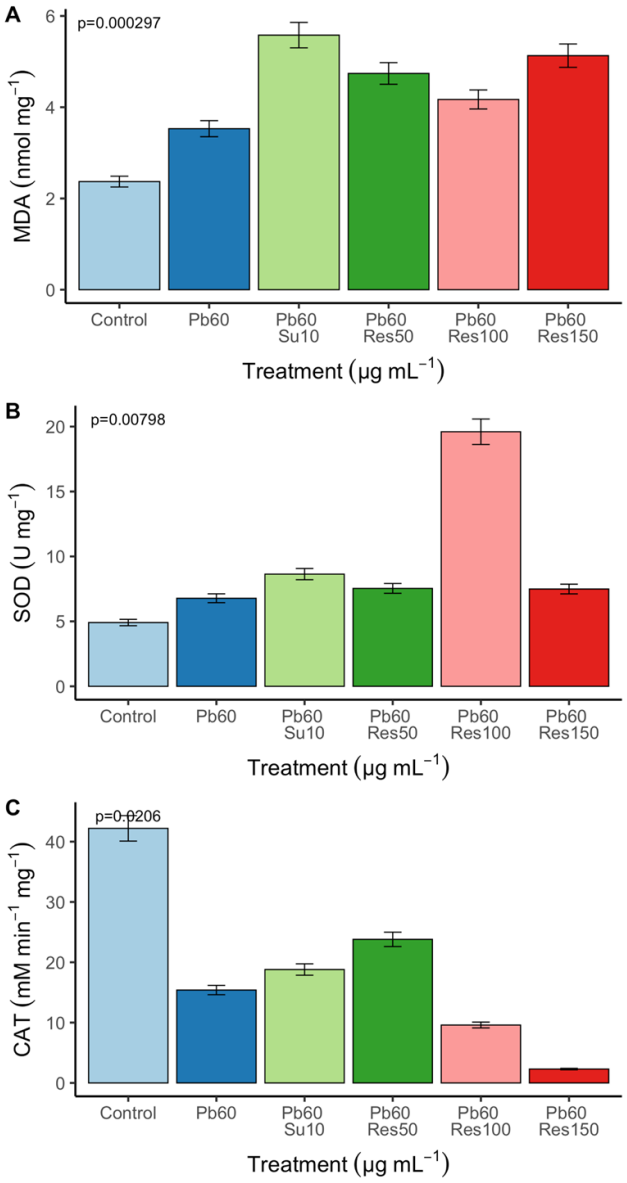

Fig. 8: Oxidant/antioxidant status in *D. melanogaster* after 28-days of exposure to Pb, resveratrol, and succimer. The data are presented as Mean ± SEM. Significance is $p < 0.05$. Pb: lead (mg/L), Su: succimer (mg/kg), Res: resveratrol (mg/kg).

Discussion

Lead (Pb) toxicity primarily affects the molecular and cellular levels by inducing oxidative damage through lipid peroxidation and weakening the antioxidant defense systems, leading to an increase in reactive oxygen species (Romero *et al.*, 2014; Moneim, 2016; Mohamed *et al.*, 2016; Balali-Mood et al., 2021). The present investigation observed that exposure of Harwich strain adult *D. melanogaster* to 60 mg/L Pb significantly reduced the fly's egg-laying ability, eclosion rate, filial generation output, climbing ability, and life span. The investigated biological indices were significantly rescued by varying concentrations of resveratrol.

A significant reduction in the number of eggs laid (fecundity) was observed in the Pb exposed flies. This could be due to an increase in cell death. This effect was mitigated by feeding flies with 150 mg/kg resveratrol. Lead acetate exposure has been reported to have adverse effects on reproduction in *Drosophila.* A reduced number of eggs laid by female flies is one of the common effects of lead acetate exposure (Jafari et al., 2017; Akintunde et al., 2020). The hatching ability of the pupae (Eclosion rate) was significantly reduced in Pb treated group. This can be attributed to the toxic effects of the lead on developing embryos, as well as interference with metabolic processes and disruption of oxidative balance. A study by Sattar et al. (2018) found that exposure to lead acetate at a concentration of 60 ppm for 14 days led to a significant reduction in the eclosion rate of *D. melanogaster.* Similarly, a study by Vela et al. (2019) showed that exposure to lead acetate at a concentration of 200 ppm for 48 hours resulted in a significant reduction in the eclosion rate of *D. melanogaster* compared to the control group.
There was significant reduction in the number of offspring produced per generation (filial generation output) across five generations in flies exposed to lead. Reduction in filial generation output can have significant implications for the population dynamics and sustainability of the fruit flies' populations. In addition, a reduction in filial generation output can lead to a decrease in genetic diversity and a reduction in the ability of the population to adapt to changing environmental conditions. Consistent with the results from this study, Javed and Usmani (2017) reported that lead acetate caused a significant reduction in the number of filial generations produced by *Drosophila melanogaster,* with the number decreasing in a dose-dependent manner. Exposure to lead acetate showed a significant decrease in the reproductive capacity of the fruit flies. This could be due to toxicity of lead acetate, which can cause oxidative stress, DNA damage, and apoptosis. The addition of resveratrol to the diet of lead-exposed *Drosophila* significantly increased the reproductive performance of *Drosophila melanogaster* exposed to lead, which may be due to resveratrol's ability to scavenge free radicals, and prevent lipid peroxidation, which can damage reproductive tissues. Obtained results were similar to other studies (Zheng et al., 2015; Islam, et al., 2018; Sivakumar et al., 2018; Tawfik et al., 2019; Guo et al., 2020).

The climbing ability, which is often used as an indicator of neuromuscular function in *Drosophila* was reduced in flies exposed to lead. The reduction in the climbing ability could be a manifestation of a decline in the neuromuscular function caused by cellular damage. The male flies had higher climbing activities when compared with the females. A study by Tripathi et al. (2019) showed that *Drosophila* exposed to lead acetate for 7 days exhibited a significant reduction in climbing activity compared to the control group. Similarly, another study by Shah et al. (2020) found that exposure of *Drosophila* to lead for 24 hours led to a significant reduction in climbing ability when compared with the control group. A decrease in the lifespan of the flies was observed when exposed to lead

acetate, which might be as a result of oxidative stress, DNA damage, and apoptosis. In previous studies, Kumar et al. (2016) and Gul et al. (2018) reported that exposure to lead acetate reduced the lifespan of *Drosophila melanogaster*. Their findings are consistent with those of our study. The addition of 150 mg/kg and 100 mg/kg resveratrol significantly improved the climbing abilities and lifespan of *D. melanogaster* exposed to lead, likely due to its ability to protect against oxidative stress, inflammation, and reduce apoptosis (Gao et al., 2016; Abolaji et al., 2018; Muhammad et al., 2019).

Uric acid (Ur) is a metabolic byproduct of purine that is excreted by insects, including *D. melanogaster*. It is an antioxidant that can scavenge ROS and protect cells from oxidative damage (Sautin and Johnson, 2008). In this study, the level of Ur was significantly increased in all treatment groups when compared to the control, this might be a protective mechanism against oxidative stress. However, high levels of uric acid can also have negative effects on the body, such as the formation of urate crystals, which can cause gout and kidney stones (Cleophas et al., 2013). Exposure to lead was found to have increased the levels of uric acid in both larvae and adult *D. melanogaster* (Kaur et al., 2016; Arora et al., 2019). The level of Ur was significantly increased in the Pb 60 mg/kg + 150 mg/kg Res group. This could be due the ability of resveratrol to enhance the activity of xanthine oxidase, an enzyme involved in uric acid synthesis (Liu et al., 2017). Consistent with the result of this study, Mishra et al. (2018), reported that treatment with resveratrol significantly increased the level of uric acid in *D. melanogaster*. The researchers suggested that this increase in uric acid could be due to the antioxidant properties of resveratrol, which may help to scavenge ROS and prevent oxidative damage in cells. The levels of creatinine, a metabolic waste product of creatine produced by the muscles and eliminated from the body through the kidneys, is also an indicator of kidney function (Bhavsar and Kottgen, 2013). Creatinine level was significantly reduced in lead exposed flies. The decrease in lead exposed flies may indicate an impaired kidney function. This is in agreement with the study of Singh et al. (2015), who also reported a significant reduction in the creatinine levels in *D. melanogaster* exposed to lead acetate, indicating kidney damage and dysfunction and contrary to the report of Arslan et al. (2022) who reported an increase in creatinine in Japanese quail exposed to lead. The differences in the classes of organism could be the reason for the observed difference. Resveratrol at 150 mg/kg significantly ($p < 0.05$) decreased the level of creatine in lead exposed flies.This implies that resveratrol may have potential protective effects against the oxidative damage caused by lead toxicity. A study by Sun et al. (2017) found that resveratrol prevented lead-induced kidney damage in rats, as evidenced by decreased levels of creatinine and other markers of kidney function.

The levels of TB and CB in treatment groups were significantly higher when compared the control. Increased bilirubin production in flies co-exposed to Pb might have been as a result of defects in hepatic uptake or conjugation, which may be as a result of destructions caused by Pb. High levels of TB and CB are generally associated with liver dysfunction and oxidative stress in various organisms. Lead acetate exposure has been shown to induce oxidative stress in *D. melanogaster* resulting in increased production of ROS and damage to cellular biomolecules. This oxidative stress can lead to liver damage and dysfunction, which may contribute to elevated levels of TB and CB. Kasperczyk et al. (2014) and Chen et al. (2017) also reported an increased level in TB and CB in rats and *D. melanogaster* exposed to lead respectively. The co-treatment of resveratrol

at a concentration of 150 mg/kg significantly reduced the total and conjugated bilirubin, suggesting a protective effect on the fat bodies of the fruit flies. This is similar to the findings of Khodayar et al. (2018) on in rats exposed to lead and treated with resveratrol.

The level of AST was significantly increased in flies exposed to lead ($p < 0.05$). Aspartate aminotransferase is an enzyme found mainly in the liver, heart, and skeletal muscles, and is involved in amino acid metabolism. Similar to the finding of this study, Nwobi et al. (2018) reported a significant increase in the levels of AST in flies exposed to 20, 40, and 60 ppm of lad acetate for 28 days. The increased level of AST may indicate hepatocellular injury leading to leakage of the enzyme into the bloodstream. The addition of 10 mg/kg Su and 100 mg/kg res significantly reduced the level of AST, suggesting a protective role. Rehman et al. (2018) investigated the protective effects of resveratrol on lead-induced hepatotoxicity in rats. The result showed that treatment with resveratrol significantly reduced the levels of AST in lead-exposed rats. Alkaline phosphatase (ALP) is an enzyme that plays an important role in various metabolic processes, including bone mineralization, liver function, and DNA synthesis. The enzyme is also considered as a marker for liver function in insects. When exposed to lead acetate, *Drosophila melanogaster* showed significant decrease in the level of ALP. This decrease in ALP activity may be attributed to lead-induced oxidative stress, which can lead to lipid peroxidation and damage to cell membranes. Several studies have demonstrated the inhibitory effect of lead on ALP activity in different organisms, including *D. melanogaster* (Kaya et al. 2017; Salehi et al., 2021). Resveratrol at 150 mg/kg significantly mitigated the effect of lead by increasing the level of ALP. This suggests that resveratrol may improve liver function, which is corroborated by several studies (Dkhil et al., 2015; Huang et al., 2015; Lin et al., 2021). A significant increase in co-enzyme Zn was observed in the Pb treated group, with the co-treatment group of 10 mg/kg Su having the highest level of Zn when compared to the control. Zinc has been reported to play a protective role against lead toxicity in various organisms, including insects (Goyer, 1997; Kazi et al., 2008; Lyu et al. 2019; Wang et al., 2019).

The electrolytes, Na ($p > 0.05$) and Cl ($p < 0.05$) were increased in the Pb treated group when compared with the control. Sodium is an essential electrolyte that plays a critical role in maintaining proper hydration, acid-base balance, and nerve and muscle function in animals, including insects. However, an excessive sodium level in insects can cause osmoregulatory stress, resulting in increased water loss, altered feeding behavior, and reduced lifespan (Lihoreau et al., 2012). Chlorine, on the other hand, is essential for many physiological functions in insects, such as, osmoregulation (Kostal, 2011; Terhzaz et al., 2015), acid-base balance (Lee, 2010), excretion (Dow, 2009) among others. The addition of resveratrol at 100 mg/kg and 150 mg/kg significantly reduced the level of Na and Cl respectively. This is contrary to the report of Jalali-Nadoushan et al. (2021), who reported a significant increase in serum levels of chloride compared to the lead-exposed group without resveratrol treatment. Lead is known to interfere with the normal functioning of calcium ions in cells and disrupt calcium homeostasis. This can lead to various consequences, including oxidative stress and apoptosis (programmed cell death). There was reduction ($p > 0.05$) in the level of Ca exposed to lead when compared to the control group. Studies have shown that exposure to led can result in decreased levels of Ca in *D. melanogaster*, which was associated with changes in the expression of genes involved in calcium homeostasis, an increase in oxidative stress and apoptotic cell death (Tiwari et al., 2020; Golub et al., 2021). A

significant decrease was observed in the level of potassium (K) in the group exposed to Pb only. This effect was significantly rescued in the 60 mg/L Pb + 150 mg/kg res group, indicating the ameliorative role of resveratrol. Potassium is an important electrolyte in insects, involved in various physiological processes including muscle contraction, nerve function, and osmoregulation (Gupta and Kumar, 2019). This could be the reason for the significantly low climbing abilities of the fruit fly observed in this study. Our finding is similar to the study of Husain et al. (2018), who reported a significant decrease in the level of potassium in the larvae of *D. melanogaster*.

Lead acetate exposure has been shown to induce oxidative stress in *D. melanogaster*, resulting in increased levels of malondialdehyde (MDA) as a marker of lipid peroxidation (Hassan et al., 2019; Tang et al., 2020). The level of MDA was significantly higher when compared to the control but significantly lower when compared with the resveratrol treated groups ($p < 0.05$). Elevated MDA levels suggest that the cell membrane has been damaged and lipid peroxidation has occurred, which can lead to cell dysfunction and death (Ayala et al., 2014). This indicates oxidative stress-induced cellular damage was not mitigated by the addition of resveratrol. It could be that the concentration of resveratrol used in this study was not sufficient to produce an antioxidant effect on oxidative stress caused by the increase in MDA level or the exposure time was inadequate. This is contrary to many studies that have shown that resveratrol has potent antioxidant properties and can protect against oxidative stress-induced damage caused by an increased MDA (Gulhane et al., 2015; Gupta et al., 2018; Liu et al., 2019). The level of SOD significantly increased when exposed to lead alone when compared to the control group. Superoxide dismutase is a key antioxidant enzyme that plays a crucial role in protecting cells from oxidative damage by catalyzing the conversion of superoxide radicals to hydrogen peroxide and oxygen (Bowler and Montague, 2020). The increase observed in this study indicates that the fly is mounting an antioxidant response to combat the oxidative stress induced by lead exposure. A significant increase was observed in groups co-treated with resveratrol, with 60 mg/L Pb + 100 mg/kg Res recording the highest level of SOD activity. This is similar to the reports of (Matin et al. 2015; Shukla et al. 2016; Guo et al. 2020). CAT activity was significantly reduced in all treated group when compared to the control ($p < 0.05$), with 60 mg/L Pb + 150 mg/kg Res having the least level of CAT. Catalase plays a crucial role in the detoxification of hydrogen peroxide and other harmful reactive oxygen species (ROS) in cells (Kouka et al. 2021). The decrease observed may be due to the generation of ROS, an impaired antioxidant defense mechanism, and increased oxidative stress caused by lead exposure, which wasn't ameliorated by the addition of resveratrol. Similar observations on the effect of lead on the CAT level in *D. melanogaster* have been reported (Tiwari et al., 2017; Liu et al., 2019). However, the concentrations of resveratrol did not ameliorate the effect of lead on the CAT activity of the fruit flies. Possible reasons for this observation could be that the doses used in this study was high to have had a positive effect on the CAT activity (Zhao et al., 2013), or it could be that the time of exposure was not sufficient enough (Kumar et al., 2014), or the increase in SOD level observed in this study, which could potentially lead to a decrease in hydrogen peroxide levels and hence a decrease in catalase activity, as also observed by Salehi et al. (2018). The dose of lead acetate could also be reason for the reduced CAT activity. This result is however contrary to the report of Kaur et al. (2016), who reported that treating lead exposed flies with resveratrol increased the CAT activity.

Conclusion

The toxicity of lead in D. melanogaster is attributed to an imbalance between reactive oxygen species (ROS) and antioxidant defense system, leading to a decrease in various biological and biochemical parameters. Resveratrol has shown to have a potential therapeutic effect against lead-induced toxicity in Drosophila melanogaster, probably by its antioxidant and anti-inflammatory properties. These results suggest the possible use of resveratrol as an affordable and safe therapeutic agent for preventing lead toxicity in humans. The safety of resveratrol in flies indicates its potential for treating neurodegenerative, hepatic, and renal diseases. However, further studies are required to fully comprehend the mechanisms underlying the protective effects of resveratrol against lead toxicity and to evaluate its safety and efficacy as a therapeutic agent for lead toxicity.

Author contributions

Conceptualization: S.M.H and R.A.; data curation: U.F.O., M.T.J., H.M., S.M.H. and R.A.; formal analysis and investigation: U.F.O., M.T.J., H.M., S.M.H. I.S.N. and R.A. writing—original draft, S.M.H.; writing, reviewing and editing: R.A., D.M.S. and I.S.N.; supervision: I.S.N., S.M.H and R.A. Fund awardees: R.A., D.M.S. and I.S.N.

Declaration of Competing Interest

The authors declare that there are no competing interests that could have influenced the work. The funding source did not contribute to the study design, data collection, analysis, interpretation, writing of the manuscript, and publication.

Funding

The research was funded by the Tertiary Education Trust Fund (TETFUND) through Institutional Base Research (IBR).

Acknowledgement

The authors would like to express their sincere gratitude to Dr. M.A. Chia for his invaluable contribution in proofreading this article. His expertise and attention to detail greatly improved the quality of the manuscript.

References

Abolaji, Amos O. Adeola O. Adedara, Mojisola A. Adie, Marta Vicente-Crespo, Ebenezer O. Farombi. (2018). Resveratrol prolongs lifespan and improves 1-methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine induced oxidative damage and behavioral deficits in *Drosophila melanogaster*. *Biochemical and Biophysical Research Communications* 503 (2018) 1042e1048.

Adedara IA, Klimaczewski CV, Barbosa NB, Farombi EO, Souza DO, Rocha J. B. (2015). Influence of diphenyl diselenide on chlorpyrifos-induced toxicity in Drosophila melanogaster. *Journal Trace Elem Med Biol*; 32:52–9.

Aebi, H. (1984). Catalase in vitro, Methods Enzymol. 105 121e126.

Akintunde, J.K., et al. (2020). Assessment of Lead Acetate-Induced Toxicity in Drosophila melanogaster. Biological Trace Element Research, 193(1), 142-151.

Arora, S., Singh, S., Datta, S., & Manna, S. K. (2019). Lead toxicity in Drosophila melanogaster: Evaluation of oxidative stress markers and DNA damage. Environmental Science and Pollution Research International, 26(4), 3364-3374. https://doi.org/10.1007/s11356-018-04048-w).

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, B.M. and Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: Mecury, Lead, Chromium, Cadmium, and Arsenic. *Front. Pharmacol.* 12.643972. doi:10.3389/fphar.2021.643972

Baur, J. A, Pearson, K. J, Price, N. L, Jamieson, N. L, Lerin, C, Kalra, A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*; 444:337–42.

Benford, D. J, Hanley, A. B, Bottrill, K, Oehlschlager S, Balls M, Brance F, Castegnara JJ, Descotes J, Hemminiky K, Lindsay D, Schilter B. (2000). Biomarkers as predictive tools in toxicity testing: the report and recommendations of ECVAM workshop 40. Altern Lab Anim; 28:119–31.

Bhavsar, N. A., and Köttgen, A. (2013). The Role of Cystatin C in Kidney Disease. Current Opinion in Nephrology and Hypertension, 22(5), 471–476. https://doi.org/10.1097/MNH.0b013e328363f599

Bier, E. (2005). Drosophila, the golden bug, emerges as a tool for human genetics. Nat Rev Genet; 6:9–23.

Bowler, C., and Montague, P. (2020). Chapter 2 - Oxidative Stress in Neurodegeneration: Mechanisms and Therapeutic Opportunities. In T. L. Suhail (Ed.), Antioxidant Enzymes (pp. 9-32). Academic Press. https://doi.org/10.1016/B978-0-12-818781-1.00002-0

Burki, T. (2020). Report says 815 million children have high blood lead levels. Lancet (London, England), 396 (10248), 370. https://doi.or/10.1016/30140-6736(20) 3168-6.

Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. (2002). Plant foods and herbal sources of resveratrol. *Journal of Agric Food Chem*; 50: 3337–40.

Chen, Z., Li, Y., Xu, X., Xu, H., Liu, C., and Liang, X. (2017). Lead exposure induces oxidative stress and hepatic dysfunction in Drosophila melanogaster. Environmental toxicology, 32(6), 1951-1961. doi: 10.1002/tox.22409

Cheng, D., Li, H., Zhou, J. P., Wang, S. (2019). Chlorogenic acid relieves lead-induced cognitive impairments and hepato-renal damage via regulating the dysbiosis of the gut microbiota in mice. *Food Funct.* 10(2):681–690. https://doi.org/10.1039/c8fo01755g.

Cleophas MC, Crișan T.O, Oostrom M, et al. (2013). The role of uric acid in the pathogenesis of human cardiovascular disease - renal and cardiovascular implications. Curr Med Chem. 2013;20(33):4133-4143. doi: 10.2174/0929867311320330003

Dow, J. A. (2009). Insights into the Malpighian tubule from functional genomics studies. Journal of Experimental Biology, 212(3), 435-445.

Ferrero, M. E. (2016). Rationale for the successful management of EDTA chelation therapy in human burden by toxic metals. *Biomed research international*, 2016, 8274504. https://doi.org/10.1155/2016/8274504.

Flora, G., Gupta, D., Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdiscip. Toxicol.* 5, 47-58

Gao, Y., Li, C., Yin, J., Shen, J., & Wang, Y. (2016). Resveratrol mitigates the oxidative stress mediated by lead toxicity in Drosophila melanogaster. Ecotoxicology and environmental safety, 131, 144-152.

Goyer, R. A. (1997). Toxic and essential metal interactions. Annu. Rev. Nutr., 17, 37-50.

Gul, S., Ahmad, S., Ashfaq, M., & Iqbal, M. (2018). Lead acetate induces oxidative stress and reduces lifespan in Drosophila melanogaster. Ecotoxicology and Environmental Safety, 157, 312-317.

Gulhane, M., Ahire, M., Hasan, M., and Chaudhari, P. (2015). Effect of resveratrol on oxidative stress and cognitive impairment induced by lead exposure in mice. Pharmacognosy magazine, 11(Suppl 1), S39.

Gupta, S.C., Tyagi, A.K., Deshmukh-Taskar, P., Hinojosa, M., Prasad, S., and Aggarwal, B.B. (2018). Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. Arch. Biochem. Biophys. 646, 36–46.

Gupta, A., and Kumar, P. (2019). Importance of potassium in insect physiology and plant-insect interactions. Journal of Plant Interactions, 14(1), 211-221.

Guo, X., Huang, Y., Zou, X., Xu, P., Zeng, F., and Li, T. (2020). Protective effect of resveratrol on lead-induced toxicity in Drosophila melanogaster. Environmental Science and Pollution Research, 27(1), 714-721.

Hassan, I.M., Al-Ghamdi, A.A., Al-Misned, F.A., Abdel-Maksoud, M.A., Abdel-Moneim, A.M., and Ali, H.M. (2019). Cadmium and lead induced oxidative stress, DNA damage, and apoptotic cell death in Drosophila melanogaster. Environmental Science and Pollution Research, 26(6), 5756-5765.

Husain, N., Siddique, Y. H., Ahmad, E., and Afzal, M. (2018). Toxic effects of lead on developmental stages of Drosophila melanogaster: Influence on survival, morphology, behavior and acetylcholinesterase activity. Chemosphere, 209, 105-114.

IHME, (2017). Global, regional, and national disability-adjusted life years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390, 1260–1344.

Jafari, M., et al. (2017). Lead acetate-induced toxicity and the protective role of curcumin in the Drosophila model: A possible model to study metal-induced nephrotoxicity and the ameliorating role of antioxidants. Journal of Trace Elements in Medicine and Biology, 44, 15-22.

Jalali-Nadoushan, M. R., Abdi, A., Rashedinia, M., and Moslemi, F. (2021). Protective effect of resveratrol on lead acetate-induced hepatotoxicity in rats: Involvement of antioxidant and anti-inflammatory mechanisms. Human & Experimental Toxicology, 40(3), 400-411.

Javed, S., and Usmani, N. (2017). Lead induced dose dependent mortality and reproductive dysfunctions in Drosophila melanogaster. Environmental pollution (Barking, Essex : 1987), 223, 321–329. https://doi.org/10.1016/j.envpol.2017.01.021

Kathura, P. (2020). Lead toxicity medication. https://emedine.medscape.com/article/1174752-medication. Retrieved 16th November 2022

Kasperczyk, S., Dobrakowski, M., Kasperczyk, A., Pawlas, N., and Birkner, E. (2014). Effects of lead exposure on the level of total bilirubin and its fractions. International journal of environmental research and public health, 11(3), 2843-2852.).

Kaur, R., Kaur, K., Singh, K., & Kaur, S. (2016). Toxicity of lead acetate on Drosophila melanogaster: Biochemical and genotoxic effects. Journal of toxicology. https://doi.org/10.1155/2016/6172937).

Kaya, H., Kucukkomurcu, S., and Bakan, N. (2017). Protective effect of lycopene against lead acetate-induced inhibition of alkaline phosphatase activity in rats. Biological trace element research, 175(2), 439-446.

Kazi, T. G., Afridi, H. I., Kazi, N., Jamali, M. K., Arain, M. B., Jalbani, N.,... and Shah, A. Q. (2008). Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biological Trace Element Research, 122(1), 1-18.

Khan, M., Park, S., Kim, H., Lee, K., Kim, H.D., Baek, S. and Hong, S. (2019). The resveratrol rice DJ526 Callus significantly increases the lifespan of *Drosophila* (Resveratrol Rice DJ526 for Longevity). *Nutrients*, 11:983. Doi: 10.3390/nu11050983

Khodayar, M.J., Seyedhosseini, F.S., Hashemnia, M., and Kheiripour, N. (2018). Resveratrol reduces lead-induced liver injury in rats. Drug and Chemical Toxicology, 41(1), 103-109. doi: 10.1080/01480545.2017.1315429

Koštál, V. (2011). Eco-physiological phases of insect diapause. Journal of Insect Physiology, 57(11), 1497-1509.

Kouka et al. (2021). Comparative study of the antioxidant activities and polyphenolic contents of five Nigerian medicinal plants. Journal of Traditional and Complementary Medicine, 11(5), 455-463. https://doi.org/10.1016/j.jtcme.2020.12.007

Kumar, A., Kaundal, R. K., Iyer, S., & Sharma, S. S. (2014). Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy. Life sciences, 95(2), 63-71.

Kumar, P., Sharma, G., and Singh, S. (2016). Lead acetate exposure induces oxidative stress and decreases lifespan in Drosophila melanogaster. Environmental Science and Pollution Research, 23(8), 7576-7583.

Lee, R. E. (2010). Principles of insect low temperature tolerance. Insects at low temperature (pp. 17-46). Springer, Dordrecht.

Li, Y., Lv, H., Xue, C., Dong, N., Bi, C. and Shan, A. (2020). Plant polyphenol: potential antidotes for lead exposure. *Biological trace element research*, 10.1007/s12011-020-02-498-w.

Lihoreau, M., Buhl, J., Charleston, M. A., Sword, G. A., Raubenheimer, D., and Simpson, S. J. (2012). Nutritional ecology beyond the individual: a conceptual framework for integrating nutrition and social interactions. Ecology letters, 15(8), 224-234.

Liu, Y., Li, H., and Sugiura, N. (2019). Resveratrol attenuates oxidative stress-induced intestinal barrier injury through PI3K/Akt-mediated Nrf2 signaling pathway. J. Funct. Foods 56, 138–147.

Lyu, Y., Li, X., Li, J., Fan, Y., Liang, Y., Li, X., & Li, B. (2019). The protective effect of zinc against lead toxicity in Drosophila melanogaster. Environmental Pollution, 245, 125-131.

Mohamed, O. I, El-Nahas, A. F, El-Sayed, Y. S, Ashry, K. M. (2016) Ginger extract modulates Pb-induced hepatic oxidative stress. *Methods in Enzymology*, 77, 398–405. https://doi.org/10.1016/S0076-6879 (81)77053-8.

Muhammad, J., Ahmad, B., Aslam, B., and Rehman, N. U. (2019). Resveratrol protects against lead acetate-induced toxicity in Drosophila melanogaster. Environmental science and pollution research international, 26(36), 36622-36631.

Moneim A. E. A. (2016) Indigofera oblongifolia prevents Lead acetate-induced hepatotoxicity, oxidative stress, fibrosis and expression of antioxidant gene transcripts in rat liver. *Pharm Biol* 54(7):1164–1172. https://doi.org/10.3109/13880209.2015.

Nwobi, G. C., Alisi, C. S., and Omeodu, C. O. (2018). Evaluation of the toxic effects of lead on the lifespan, antioxidant status, and oxidative stress biomarkers in Drosophila melanogaster. Journal of toxicology. https://doi.org/10.1155/2018/5458060

Obeng-Gyasi E. (2018). Lead exposure and oxidative stress – a life course approach in U.S. adults. Toxics; 6:42.

Obeng-Gyasi, E., Armijos, R. X., Weigel, M. M., Filippelli, G. M., Sayegh, M. A. (2018). Cardiovascular-related outcomes in US adults exposed to lead. *Int J Environ Res Public Health*; 15:759.

Pastor RF, Restani P, Di Lorenzo C, Orgiu F, Teissedre PL, Stockley C, Ruf JC, Quini CI, Garcı`aTejedor N, Gargantini R, Aruani C, Prieto S, Murgo M, Videla R, Penissi A, Iermoli RH. (2019). Resveratrol, human health and winemaking perspectives. *Crit Rev Food Sci Nutr*; 59:1237–55.

Rehman, K., Fatima, F., Waheed, I., Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of Cell Biochemistry*, 119(1):157–184. https://doi.org/10.1002/jcb.26234

Salehi, B., Martorell, M., Motilva, V., and Sureda, A. (2021). Protective effects of saffron (Crocus sativus L.) and its bioactive constituents against oxidative stress-induced disruptions in biological systems: A review. Journal of Agricultural and Food Chemistry, 69(3), 759-773.

Sattar, S., Jahan, S., Yasmin, A., Fatima, B., and Yousuf, S. (2018). Impact of lead acetate on developmental stages of Drosophila melanogaster. Toxicology Reports, 5, 1114-1121.

Sautin, Y. Y., and Johnson, R. J. (2008). Uric acid: the oxidant-antioxidant paradox. Nucleosides, Nucleotides and Nucleic Acids, 27(6-7), 608-619. doi: 10.1080/15257770802138558.

Shah, P., Yadav, K., Bhattacharya, R., Gupta, R. K., and Shukla, R. K. (2020). Protective effect of genistein against lead-induced neurotoxicity in Drosophila melanogaster. Journal of applied toxicology: JAT, 40(10), 1371-1379.

Staats, S., Wagner, E.A., Kowalewski, B., Rieck, T.F., Soukup, T.S., Kulling, E.S. and Rimbach, G. (2018). Dietary resveratrol does not affect life span, body composition, stress response, and longevity-related gene expression in *Drosophila melanogaster*. *Int. J. Mol. Sci.* 19 (1): 223. https://doi.org/10.3390/ijms19010223

Sivakumar, S., Sivasubramanian, A., and Senthil-Nathan, S. (2018). Resveratrol mitigates lead-induced reproductive toxicity in male Drosophila melanogaster. Environmental science and pollution research international, 25(18), 17872-17880.

Tawfik, W. A., El-Sayed, Y. S., El-Gendy, K. S., and Abd El-Aziz, R. M. (2019). Protective effect of resveratrol against developmental toxicity of lead in Drosophila melanogaster. Environmental Science and Pollution Research, 26(5), 4455-4462.

Tang, M., Zhang, Y., Li, C., Yao, H., and Zhang, X. (2020). Drosophila as a model to study lead toxicity: mechanisms of lead-induced oxidative stress and apoptosis. Environmental Science and Pollution Research, 27(35), 44340-44351.

Terhzaz, S., Cabrero, P., Brinzer, R. A., Halberg, K. A., Dow, J. A., and Davies, S. A. (2015). Insect osmoregulation. Annual Review of Entomology, 60, 277-299.

Tripathi, A., Singh, A., and Kumar, A. (2019). Ameliorative effect of dietary sesame oil on lead-induced toxicity in Drosophila melanogaster. Comparative biochemistry and physiology. Part C, Toxicology & pharmacology, 219, 14-21.

Tsai, M., Huang, S. and Cheng, S. (2017). Lead Poisoning Can Be Easily Misdiagnosed as Acute Porphyria and Nonspecific Abdominal Pain. Case Rep. Emerg. Med.; 2017: 9050713. doi: 10.1155/2017/9050713

sVela, F. E., González-Morales, N., Muñoz-Sánchez, V. R., and Ostrosky-Wegman, P. (2019). Toxic effects of lead and cadmium on the developmental stages of Drosophila melanogaster. Journal of Toxicology, 2019.

Wang, Q., Wu, Q., Feng, W., and Ye, S. (2019). Protective effects of zinc on lead-induced oxidative stress and DNA damage in Drosophila melanogaster. Journal of Environmental Sciences, 75, 69-77.

Yeh, C. B, Hsieh, M. J, Lin, C. W, Chiou, H. L, Lin, P. Y, Chen, T. Y, Yang, S. F. (2013). The anti-metastatic effects of resveratrol on hepatocellular carcinoma through the down regulation of a metastasis associated protease by SP-1 modulation. PLoS One; 8: e56661.

Zheng, X., Zhu, J., Zhang, S., Wu, S., Chen, J., and Wang, J. (2015). Resveratrol attenuates the deleterious effect of lead on Drosophila melanogaster. Ecotoxicology, 24(9), 1922-1930.

Zhao, J., Dong, X., Hu, X., Long, Z., Wang, L., Liu, Q., ... and Ma, Y. (2013). Resveratrol attenuates oxidative stress-induced intestinal barrier injury through PI3K/Akt-mediated Nrf2 signaling pathway. Oxidative medicine and cellular longevity, 2013.