

Taurine Reduced Reproductive Performance and DNA Damage Induced by Lead in *Drosophila melanogaster*Fahriye Zemheri-Navruz^{1,a,*}, Ozge Celiktas-Kostekci^{2,b}, Sinan Ince^{3,c}¹Bartın University, Faculty of Science, Department of Molecular Biology and Genetics, Bartın, Türkiye²Bartın University, Institute of Science, Bartın, Türkiye³Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Afyonkarahisar, Türkiye^aORCID: 0000-0003-1744-1091; ^bORCID: 0000-0001-9762-9144; ^cORCID: 0000-0002-1915-9797

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Abstract

Lead is one of the most important pollutants in the environment and food chain. This heavy metal causes serious health risks, especially cancer, in humans and animals. Taurine is an amino acid that can be synthesized mainly from methionine and cysteine and is used especially in the food industry. This study investigated the possible protective role of taurine on reproductive performance and DNA damage in *Drosophila melanogaster* exposed to lead. Lead and taurine were added to the broth of *D. melanogaster* at 100 µM and 3 mM, respectively, for 15 days. The Comet method was used for the determination of DNA damage. It was found that there was raise in DNA damage in the lead-administered groups, whereas taurine reduced the DNA damage induced by lead. In addition, it was determined that lead caused a decrease, whereas taurine had a positive effect on reproductive performance. As a result, it was determined that taurine prevented the negative effects of lead on *D. melanogaster* and showed protective properties.

Keywords: Model organism, lead, taurine, pupae and reproductive development, comet assay.**INTRODUCTION**

Lead, a heavy metal, is found in the environment, food, and industrial products including paints, batteries, gasoline, and cosmetics. Lead is one of the most dangerous chemicals for human and animal health (Kumar et al., 2020). Lead shows its toxicity on the blood circulatory, excretory, and nervous systems, and can cause adverse effects that can lead to anemia, nervous system disorders, kidney and liver damage, hearing impairment, gastrointestinal damage, Alzheimer's disease, cancer and progression of cancers (Mani et al., 2019). Activation of signaling pathways involving c-Jun NH2-terminal kinase, phosphoinositide 3-kinase, Akt, and p38 mitogen-activated protein kinase plays a crucial role in the pathophysiology of lead toxicity. Lead exposure is known to enhance apoptosis and severely disrupt cellular differentiation and maturation processes (Singh et al., 2018). In addition, *in vitro* studies in mammalian cells show that lead compounds can cause different genotoxic effects such as aneugenicity (Thier et al., 2003), clastogenicity (Bonacker et al., 2005), and single-stranded DNA breaks (Pasha Shaik et al., 2006). It is known that in addition to those taken in the diet, antioxidants taken from outside play an important role in maintaining the oxidative balance and/or against oxidative damage (Brenneisen et al., 2005). Pharmacologically active substances such as ascorbic acid, alpha tocopherol, and retinol are considered to be the strongest antioxidants that cut free radicals and

thus oxidative chain reactions. Studies have also shown that antioxidants prevent the formation of free radicals resulting from oxidative damage and contribute to the maintenance of cell viability (Bahadorani et al., 2008).

Taurine is 2-aminoethanesulfonic acid and is obtained from cysteine and methionine amino acids (Jong et al., 2012). Taurine has a protective effect on the cell by preventing mitochondrial membrane permeability and apoptosis. However, it also plays a role in neuromodulation, autophagy, and osmoregulation events (Chan et al., 2013; Bai et al., 2016). In a study evaluating the effects of caffeine and taurine on *D. melanogaster*, it was reported that high-dose taurine increased the effect of caffeine and reduced its effect at a low dose (Lin et al., 2010). In addition, taurine has been found to prevent aging and increase longevity in *D. melanogaster* (Yang et al., 2012).

D. melanogaster has in a very short time and rapid reproduction rate and is a frequently preferred model organism for elucidating the molecular mechanisms of genotoxicity, oxidative stress, and various diseases (Kreipke et al., 2017). The aim of this study was to investigate the potential protective effect of taurine on *D. melanogaster* exposed to lead. For this purpose, reproductive performance and pupae numbers of *D. melanogaster* were determined as a result of lead and taurine applications. DNA damage resulting from the applications was evaluated by Comet analysis.

MATERIALS AND METHODS

D. melanogaster cultures were grown in a 60-70% humidity refrigerated incubator set at a constant temperature of $24 \pm 1^\circ\text{C}$ under laboratory conditions. In preparation of the medium, brewer's yeast (9 g), corn flour (104 g), sugar (94 g), agar (6 g), distilled water (1020 ml), acid mixture at 6 ml (including 8.36 ml propionic acid, 7.83 ml orthophosphoric acid, and 1081 ml distilled water) were used.

D. melanogaster experimental groups were divided into 4 groups; control, lead (100 μM), taurine (3 mM), and lead plus taurine administration groups. The dose of lead (Lead-II acetate trihydrate; CAS number: 6080-56-4, Sigma-Aldrich, St. Louis, MO, USA) used in the study Morley et al., (2003) and the dose of taurine (Taurine; CAS number: 107-35-7, Sigma-Aldrich, St. Louis, MO, USA) were based on the amounts stated by Arslan-Acaroz et al (2020) and Besson et al., (2005). For the assessment of reproductive performance and pupae numbers in *D. Melanogaster*, 50 third-stage larvae were added to each of the four group culture flasks. Larval development was monitored 2ort , and the number of male and female flies was recorded for 15 days (Table 1).

DNA damage was also determined by Comet analysis. For each group, 10 unmated females and males adult were added in 50 ml medium including lead and taurine to growth for 15 days. The comet assay is a technique used to detect DNA single-strand breaks. In this study, fly tissue was torn into small pieces and dispersed between two layers of agarose gel on microscopic slides. The bottom layer consisted of normal melting agarose (1.0%), while the upper layer was made of low melting agarose (0.5%). After polymerization of the gel at 4°C for 5 minutes, the slides were immersed in a lysis buffer containing 2.5 M NaCl, 10 mM Tris base, 100 mM EDTA, pH 10, 10%

dimethyl sulfoxide (DMSO), and 1% Triton X-100 for 1 hour at 4°C . To induce DNA unwinding, the slides were placed in an electrophoresis buffer containing 10N NaOH and 200 mM EDTA, pH>13.0, for 15 minutes, and then subjected to electrophoresis (24 V and 300 mA) for 40 minutes. Afterward, the slides were placed in a 0.4M Tris buffer at room temperature for neutralization. Ethidium bromide (10%) was used to stain the slides, which were then examined under fluorescent microscopy (Zeiss, Germany). (Dhawan et al., 2003). A total of 100 cells were scored per slide in triplicate, and all steps were carried out under minimal illumination. The extent of DNA damage was evaluated based on the size of the comet tail, with a value of 0 indicating undamaged DNA and values of 1 to 4 indicating increasing levels of damage. The scoring was performed separately for the abdomen tissue of the flies (Olive and Banáth, 2006). The experiments were replicated three times each.

At the end of the study, the data obtained for reproductive performance in *D. Melanogaster* were expressed as numbers and percentages. In addition, one-way ANOVA analysis of variance (Graphpad prism 8.0) was used to evaluate the DNA damage findings (Mean $\pm$ SEM) between groups, and Dunnet's multiple comparisons test was used to evaluate the significance between groups. P-value of <0.05 was considered statistically significant.

RESULTS

In the study, it was determined that lead decreased reproductive performance and pupae development in *D. Melanogaster*. On the other hand, it was determined that reproductive performance and pupae development increased in *D. Melanogaster* when compared to the lead-administered group as a result of the administration of taurine plus lead (Table 1).

Table 1. The effect of taurine on reproductive performance and pupae development in lead-administered *D. Melanogaster*.

Groups	Adult				Pupae		
	Female	%	Male	%	Total	Development	%
Control	51	100	62	100	113	174	100
Lead	24	47.05	23	37.09	47	101	58.04
Taurine	48	94.11	60	96.77	108	166	95.40
Lead+Taurine	40	78.43	42	67.74	82	152	87.35

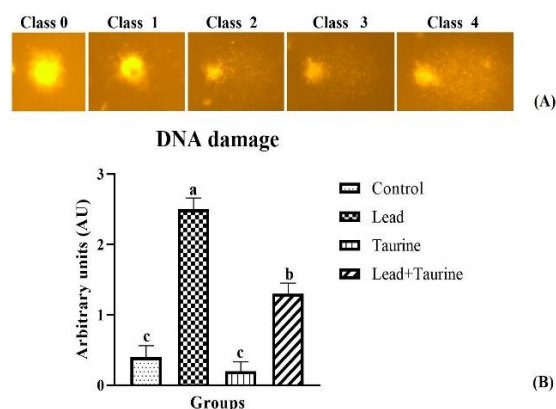


Figure 1. (A) They represent classes 0–4 as used for visual scoring (Arbitrary units (AU)). (B) The effect of taurine on DNA damage in lead-administered *D. melanogaster*. Values with different letters are statistically significant ($P<0.05$).

DNA damage in *D. melanogaster* was found to be higher in the lead-administered group compared to the control group ($p<0.0001$). On the other hand, taurine was found to reduce lead-induced DNA damage ($p<0.001$). Reproductive performance, pupae development, and DNA damage were found to be close to the control group in the taurine groups (Figure 1).

DISCUSSION AND CONCLUSION

Lead exhibits a negative effect during the developmental period and is considered a significant risk factor as it markedly alters reproductive development resulting in biochemical and physiological changes (Chon et al., 1992). Olakkaran et al., (2018) reported that 0.2, 0.4, 0.6, and 0.8 mM lead in a concentration-dependent were induced biochemical (increased reactive oxygen radicals and decreased antioxidant status) and genetic (increased in the mutant spots per wing and DNA damage) effects in *D. melanogaster*. Similarly, lead administration at doses of

0.2, 0.4, 0.6, and 0.8 mM caused oxidative stress and DNA damage by directly binding to DNA whereas decreased antioxidant status (Shilpa et al., 2021). Additionally, lead exposure in different doses (0.125, 0.25, 0.5, 0, and 02 mg) was evaluated on external morphology in *D. melanogaster* at 48 hours post-treatment. It was observed that lead caused abnormalities and deformities developed in the larvae of flies. Elongated and de-shaped wings, elongated and folded legs, and change in color of larvae, pupae, and adults were also observed (Haq et al., 2011). Safaee et al (2011) reported that different concentrations of lead-ion (20-300 mg/L) were added to the culture for eight hours and lead increased larvae and pupae periods, but decreased the conversion rate of larvae to pupa, pupa to adult, and eggs hatching. It also reduced the growth rate of larvae length/ width, pupa length/width, and adult length. Hirsch et al., (2003) reported that Canton-S flies were grown from eggs to adults (6-7 days) in a medium containing lead acetate solution (2-100 µg/g). At the end of the study, they reported that 50 µg/g lead acetate reduced fertility, and locomotor activity was found to be low in male flies. In another study, it was reported that exposure to 2 mM lead had growth retardation, decreased survival rate, impaired motility, and decreased egg production in *D. melanogaster* (Liu et al., 2020). Similarly in these studies, this study showed that lead exposure decreased reproductive performance together with pupae development, and caused DNA damage in *D. melanogaster*.

Many studies stated that antioxidant substances have a protective effect against the harmful effects of chemical agents on *D. melanogaster*. Jimenez-Del-Rio et al., (2010) reported that 0.1-1 mM polyphenols (gallic acid, epigallocatechin, caffeic acid, propyl gallate, coumaric acid, ferulic acid, epicatechin, and epigallocatechin gallate) improved movement activity induced by paraquat in *D. melanogaster*. Similarly, (-)- α -bisabolol (5, 25, and 250 µmol/L) with anti-inflammatory and antibacterial properties has been reported to have a protective effect against rotenone (500 µmol/L)-induced motor function loss and oxidative stress in *D. melanogaster* (Leite ve ark. 2018). Nagpal and Abraham, (2017) stated that β -carotene (0.25, 0.5, and 1%) and tea polyphenol (1%), which have known antioxidant properties, have a protective effect against 10 Gy γ -radiation-induced sex-linked recessive lethal mutation and oxidative stress in *D. melanogaster*. Moreover, some researchers reported that taurine could enhance the life span of *D. melanogaster* (Suh et al., 2017) and reduce the harmful effect of *C. Elegans* (Kim et al., 2010). Likewise, in the present study, taurine reversed the lead-induced harmful effects in *D. melanogaster*.

In conclusion, taurine regarding its antioxidant effect inhibited the negative effects of lead on reproductive performance, pupa development, and DNA damage in *D. melanogaster*.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship contributions

Concept: S.I., F.Z.N, Design: S.I., F.Z.N., Data Collection or Processing: F.Z.N, O.C.K., Analysis or Interpretation: S.I., F.Z.N, O.C.K., Literature Search: S.I., Writing: S.I., F.Z.N.

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