*J. Appl. Biol. Sci.*, 19 (2): 81-87, May 2025
https://dx.doi.org/10.71336/jabs.1438
RESEARCH ARTICLE

Journal of Applied Biological Sciences
EISSN: 2146-0108; License: CC BY 4.0
Anatolia Academy of Sciences
Open Access

# Protective effects of *Satureja hortensis* on DNA damage and reproduction performance in lead-exposed *Drosophila melanogaster*

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Received: December 18, 2024
Revised: April 29, 2025
Accepted: May 02, 2025

**ABSTRACT:** Lead is considered one of the most dangerous heavy metals for human health and can be found in the environment, as well as in food and products. *Satureja hortensis*, commonly known as savory, is a plant used to treat various diseases in folk medicine. This study aims to determine the effects of the ethanol extract of *S. hortensis* on reproductive performance and DNA damage in lead-exposed *Drosophila melanogaster*. The plant's ethanol extract was obtained using the maceration method. After applying the extract and lead to *D. melanogaster* experimental groups for 15 days, their reproductive performance (adult female and fly formation, as well as pupal counts) and DNA damage levels were investigated using the Comet assay. The results showed that lead reduced reproductive performance and pupal development in *D. melanogaster* and caused DNA damage. However, when *S. hortensis* extract was applied together with lead, reproductive performance and pupal development in *D. melanogaster* increased quantitatively, while DNA damage decreased. In conclusion, *S. hortensis* exhibited a protective effect in lead-exposed *D. melanogaster* and can play an antigenotoxic role against lead and other metal.

**KEYWORDS:** *Satureja hortensis*, *Drosophila melanogaster*, lead, reproductive performance, DNA damage.

## INTRODUCTION

Turkey, which is one of the countries rich in plant diversity, has a wide range of medicinal and aromatic plants. Many of these plants are used in the treatment of diseases and the production of medicines. In addition to their use for medicinal purposes by the public, plants are widely used in the food, spice, resin, volatile insecticide, dye, and cosmetic industries [1]. While many *Thymus*, *Origanum*, *Satureja*, *Coridothymus*, and *Thymbra* species, which are known as oregano in Turkey, are collected from nature, some are also grown through agriculture [2]. The Satureja hortensis plant is known as "çibriska", "çubriza", "zater", "sater", or "geyikotu" in Turkey [3]. *S. hortensis* is distributed in rocky and eroded sloping areas of the Marmara region (Istanbul, Sakarya), Western and Central Black Sea (Zonguldak, Amasya, Samsun), Central Anatolia (Ankara, Nevşehir, Sivas), and Eastern and Southeastern Anatolia (Erzincan, Adıyaman, Diyarbakır, Erzurum). It is an annual plant that can grow up to 30-35 cm tall with **well-**developed lateral branches. Its flowers are white, purple, or pink [4]. The flowers, leaves, and roots of *S. hortensis* are used for alternative treatment purposes for various diseases or complications such as digestive system cramps, nausea, diarrhea, and muscle pain [5]. The volatile oils in the plant, such as thymol and carvacrol, are present at levels above 5% and exhibit antimicrobial properties against food, plant, and human pathogens. The plant also contains compounds with pharmacological activity such as flavonoids and tannins [6, 7, 8]. Due to its content of these compounds, *S. hortensis* also exhibits antioxidant [9], anti-inflammatory, analgesic [10], and anti-hypercholesterolemic properties [11].

Lead is considered one of the most dangerous chemicals for human health, as it can be found in the environment (especially in dust, water, and soil), food, and manufactured lead products including lead-based paints, pipes, batteries, cosmetic products, gasoline, food cans, children's toys, ammunition, vitrified ceramics, and herbal remedies [12]. Lead's toxic effects on humans are mainly evident in the nervous system, blood, and kidneys, causing anemia, nervous system disorders, kidney and liver damage, hearing impairment, gastrointestinal damage, reduced IQ in children, behavioral and learning disorders, Alzheimer's disease, and negative effects that may lead to the progression of cancer such as lung cancer [13]. The activation of phosphoinositide 3-kinase, c-Jun NH2-terminal kinase, p38 mitogen-activated protein kinase, and Akt signaling pathways are important in lead's cytotoxicity. Lead increases apoptosis through signal cascades and associated factors and significantly disrupts cell differentiation and maturation. In addition, lead has a significant impact on metabolic pathways, including biosynthesis, causing anemia in individuals exposed to lead [14]. Furthermore, in vitro studies conducted on mammalian cells demonstrate that lead compounds may cause various genotoxic effects [15], clastogenicity [16], and single-strand DNA breaks [17].

Drosophila *melanogaster*, a eukaryotic organism, has a rapid reproductive rate over a short period of time. Additionally, it shows similarities to mammalian biological systems in terms of biology, physiology, and neurology [18, 19]. It is a widely used model organism in studies of oxidative stress [20], elucidation of molecular mechanisms in various diseases [21, 22], genotoxicity [23], and lifespan research related to aging [24, 25]. This study aims to assess the effects of the ethanolic extract of the *S. hortensis* plant on DNA damage and reproductive performance in *D. melanogaster* exposed to lead. For this purpose, reproductive performance and pupal numbers of *D. melanogaster* were determined after lead and plant extract applications. Additionally, DNA damage in *D. melanogaster* was evaluated by Comet analysis. The potential use of this plant's protective effects against heavy metal toxicities, as determined at the end of the study, was evaluated for its role in the development of new treatment methods against metal toxicity.

## MATERIALS AND METHODS

### Collection of the plant and preparation of the ethanol extract

The *S. hortensis* plant was collected from a garden in Kızılinler village of Eskişehir province during the flowering season in July-August 2022 (Figure 1A). After obtaining a herbarium number (AKU-9325), the collected plants were dried in the shade. After drying, the plants were ground into a powder (Figure 1B), and the ethanol extract was obtained using the maceration method. For this purpose, 25 g of dried plant material was combined with 100 ml of solvent. The mixture was kept in the dark with frequent stirring. After 24 hours, the resulting macerate was filtered through filter paper. Subsequently, the solvent was removed using a rotary evaporator to obtain a greenish-black ethanol extract (3.5%; Figure 1C). The obtained extract was stored in 15-ml Falcon tubes at -20°C until the analysis stage.

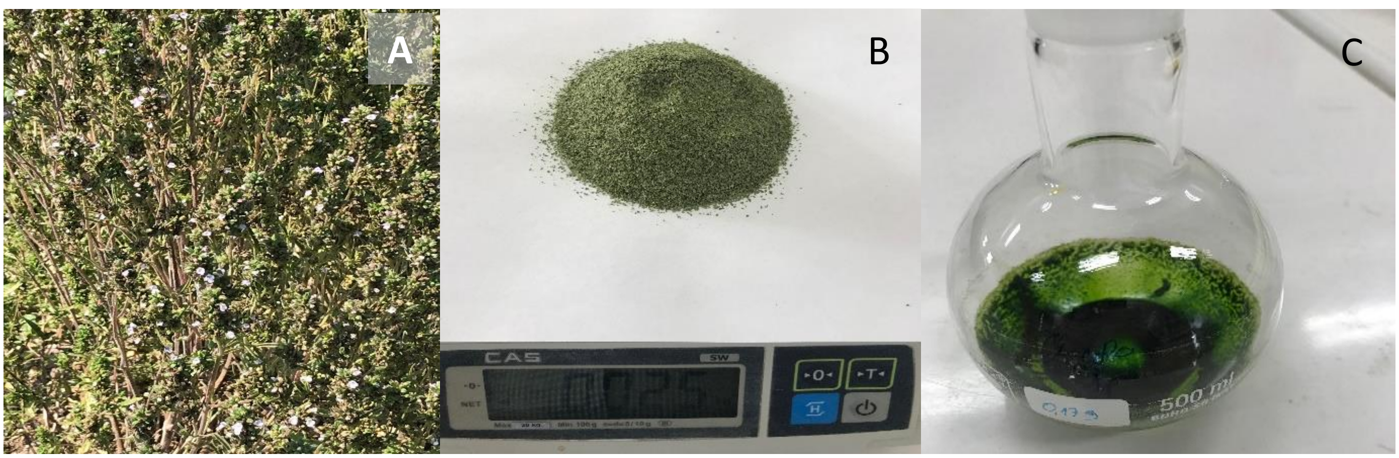


**Figure 1.** A) Flowering, B) powdered, and C) ethanol extract of S. hortensis.

### Experimental material

In this study, the Oregon-R wild type strain of *D. melanogaster*, which is genetically homozygous and not mutant, has been used from the laboratory stock of the Molecular Biology and Genetics Research Laboratory at the Faculty of Science at Bartin University since 2018. *D. melanogaster* cultures were grown in culture dishes with constant temperature (24°C) and humidity (%60-70) in a refrigerated incubator located in the laboratory. The culture dishes were made colorless and transparent for easy monitoring of the flies' development.

### Content and preparation of Drosophila melanogaster food

The food mixture was prepared using a combination of corn flour (104 g), sugar (94 g), agar (6 g), beer yeast (9 g), distilled water (1020 ml), and an acid mixture (6 ml) consisting of orthophosphoric acid (7.83 ml), propionic acid (8.36 ml), and distilled water (1081 ml) [26, 27]. For the preparation of the food, 500 ml of distilled water was mixed with corn flour, sugar, and beer yeast and thoroughly blended. In the remaining 520 ml of distilled water, agar was added and boiled until it was completely dissolved.

Meanwhile, the mixture of flour, sugar, yeast, and water was heated. The melted agar was poured onto the boiling mixture in three stages and continuously stirred. After it became homogeneous, the acid mixture was added and stirred to prevent the growth of fungi and bacteria. However, a sufficient amount of acid mixture was added to avoid excessive yeast, which could inhibit the development of flies. The prepared medium supports the rapid growth of yeast and provides a good source of nutrients for the flies. Therefore, care was taken not to add too much yeast (as it could reduce the oxygen level in the environment by using the necessary O2 for the flies). The prepared warm food mixture was poured into sterilized culture bottles in approximately 10 ml portions and closed with cotton. It was stored in a clean and cool place until flies were added after cooling [28].

**Experimental setup**

For each group, 10 male and 10 female adult *D. melanogaster* individuals were cultured in transparent culture bottles containing 10 mL of growth medium with lead acetate (Pb(CH3CO2)2•3H2O; Sigma-Aldrich, St. Louis, MO, USA) and *S. hortensis* extract for 15 days. Five experimental groups of *D. melanogaster* were created for the study, including a control group, 1% ethanol group (to see the effect of the solvent), 0.5 mg/mL *S. hortensis* ethanolic extract group (obtained from preliminary studies), and 100 µM lead group [29, 30], and the experiment was repeated three times. In preliminary experiments to determine the dose of S. hortensis plant extract, extract doses of 0.5, 1, 2.5, and 5 mg/mL were applied to *D. melanogaster* cultures, and the dose of 0.5 mg/mL, which did not show any negative effects, was used in the experimental stage.

**DNA damage analysis**

Comet analysis was performed by preparing separate slides for each group of adult *D. melanogaster* to determine DNA damage. For this analysis, low-melting point agarose (LMA) with a concentration of 0.5% was melted and kept at 40°C to prevent it from solidifying. Normal-melting point agarose (NMA) with a concentration of 1% was prepared, and slides were coated with it. Fly samples (5 individuals) were homogenized in 100 µL of Hanks's Balanced Salt (HBSS) solution, added to 100 µL of 0.5% LMA, and mixed. Then, the mixture was added to the slide coated with NMA and incubated at 4°C for 5 min. The prepared samples were treated with lysis solution (100 mM EDTA, 2.5 M NaCl, and 10 mM Tris base, adjusted to pH 10, and supplemented with freshly prepared 1% Triton X-100 and 10% DMSO) for 1 h at 4°C. The samples were then treated with electrophoresis buffer (prepared with 200 mM EDTA and 10 N NaOH to pH>13.0) at 4°C for 15 min. Alkaline electrophoresis (24 V and 300 mA) was applied for 40 min. The samples were then neutralized with Tris buffer solution (0.4 M; pH 7.5) for 5 min. After these procedures, the samples were stained with Red Safe (10 µL/mL) and examined under a fluorescence microscope (Zeiss, Germany) [31]. When examining the sample images, the damage score of 100 randomly selected cells was recorded from 0 to 4 (0—no damage, 4—very damaged), and the results were expressed in arbitrary units (AU) [32].

**Statistical analysis methods**

At the end of the study, reproductive performance (number of adults and pupae) of *D. melanogaster* were expressed numerically and as percentages. DNA damage analysis between groups was performed using GraphPad Prism Version 8 software. Group differences were evaluated using one-way analysis of variance (ANOVA), and significance between groups was evaluated using Dunnett's multiple comparison test. In addition, an independent (unpaired) t-test was used for comparison between groups treated with lead and *S. hortensis* extract combined with lead. Data were presented as mean ± standard error, and statistically significant was considered $p<0.05$.

## RESULTS AND DISCUSSIONS

The effects of lead and *S. hortensis* extract, alone and in combination, on female and male adult and pupal numbers in *D. melanogaster* are shown in Table 1. The Comet assay image of DNA damage determined by Comet analysis is shown in Figure 2, and the findings are presented in Figures 3 and 4. In the study, the lead reduced reproductive performance and pupal development in *D. melanogaster*. On the other hand, it was determined that when *S. hortensis* extract was applied together with lead, reproductive performance and pupal development in *D. melanogaster* increased quantitatively compared to the group treated with lead alone. Furthermore, it was observed that reproductive performance and pupal development in the groups treated with *S. hortensis* extract and ethanol remained close to the control group and did not have a negative effect. When DNA damage in female and male *D. melanogaster* was compared to the control group, it was found to be higher in the lead-treated group (2.40±0.22 AU and 2.85±0.26 AU) ($p<0.0001$). In contrast, when *S. hortensis* extract was administered together with lead, DNA damage was determined to be 1.75±0.14 AU in females ($p<0.0001$) and 1.70±0.21 AU in males ($p<0.0005$). This showed that *S. hortensis* extract reduced lead-induced DNA damage and exhibited antigenotoxic effects. Additionally, it was determined that DNA damage was similar to the control group in groups treated with *S. hortensis* extract alone (1.10±0.17 AU and 0.95±0.25) and ethanol (0.85±0.13 AU and 0.70±0.12 AU) in female and male *D. melanogaster*, respectively (p>0.05).

**Table 1.** Effect of *S. hortensis* extract on reproductive performance in *D. melanogaster* exposed to lead.

| Groups | Adult | | | | | Pupa | |
|---|---|---|---|---|---|---|---|
| | Female | % | Male | % | Total | Development | % |
| Control | 24 | 100 | 34 | 100 | 58 | 70 | 100 |
| *S. hortensis* extract | 23 | 95.8 | 32 | 94.1 | 55 | 68 | 97.1 |
| Lead | 15 | 62.5 | 17 | 50 | 32 | 54 | 77.1 |
| *S. hortensis* extract + Lead | 20 | 83.3 | 22 | 64.7 | 42 | 61 | 87.1 |
| Ethanol | 22 | 91.6 | 31 | 91.1 | 53 | 67 | 95.7 |

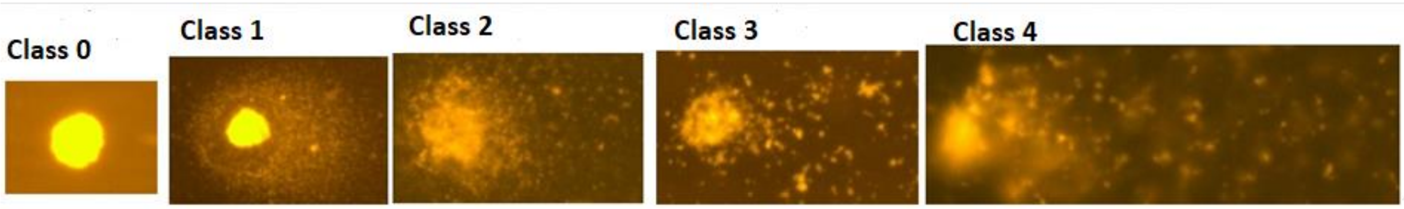


**Figure 2.** Classification of DNA damage observed in the Comet test

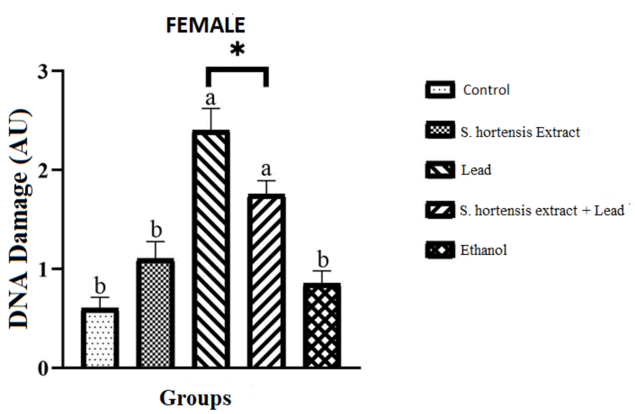


**Figure 3.** Effect of *S. hortensis* extract on DNA damage in female *D. melanogaster* exposed to lead. a,b: Values with different letters are statistically significant (p<0.05). *The difference between the groups exposed to lead alone and lead with *S. hortensis* extract is statistically significant (p=0.0186).

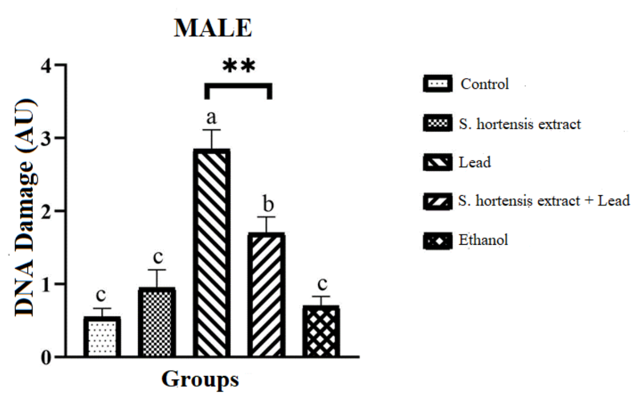


**Figure 4.** Effect of *S. hortensis* extract on DNA damage in male *D. melanogaster* exposed to lead. a,b,c: Values with different letters are statistically significant (p<0.05). **The difference between the groups exposed to lead alone and lead with *S. hortensis* extract is statistically significant (p=0.0018).

Exposure to lead is considered an important risk factor, especially during the developmental period, as it alters biochemical and physiological processes [33]. Along with medical treatment, complementary and alternative therapies are increasingly being used against toxic substances, and studies related to the use of plants for this purpose are emerging [34].

Similarly, *Drosophila* is used as a model because of its short production times, which enable the determination of the effects of toxic substances at different biological stages, including the larva, pupa, and adult forms [35]. This model helps to understand the effect of toxic substances on humans due to the presence of similar developmental pathways and stress responses [36].

Some studies have shown that lead creates genotoxicity through oxidative stress-mediated damage to the synthesis pathway in *D. melanogaster*, as well as indirect or direct damage to DNA by affecting the antioxidant system [37, 38].

In one study, the in vivo genotoxic activity of 2, 4, and 8 mM lead chloride (PbCl2) and lead nitrate (Pb(NO3)2) was investigated in *D. melanogaste*r using the Wing-spot test and Comet test for two different genetic endpoints. The results of the wing spot test showed that neither lead chloride nor lead nitrate caused significant increases in mutant spot frequency. However, the in vivo comet test in hemocytes highlighted that lead nitrate caused significant increases in DNA damage through a direct dose-response model, suggesting a genotoxic risk associated with lead exposure [39]. In a study by [40], *D. melanogaster* exposed to lead (2 mM) exhibited various health defects, including developmental retardation, decreased survival rate, impaired movement ability, and reduced egg production. Additionally, lead's immunotoxic effect on *D. melanogaster*, including a decrease in the number of hemocytes, including crystal cells, led to a decrease in phenoloxidase activity and an increase in susceptibility to *B. subtilis* [41]. Similar to the negative effects of lead observed in this study, reproductive performance was found to be reduced and DNA damage increased in *D. melanogaster* exposed to lead. Also, many studies report that females show higher resistance than males in cases of oxidant status and DNA damage [42, 43]. Similarly, the study found that gender was also effective in this toxicity, with females being more resistant than males.

When extracts obtained from the aerial parts of *S. hortensis* were examined, it was reported that the methanolic extract obtained by maceration contained higher amounts of rosmarinic acid (24.9 mg/g), caffeic acid (1.3 mg/g), naringenin (1.1 mg/g), ferulic acid (220 µg/g), and apigenin (165 µg/g). Other flavonoids (luteolin) and glycosides (apigetrin and vitexin), as well as flavonol (quercetin), flavonol glycosides (isoquercitrin, astragalin, quercitrin), and coumarin derivatives (aesculin and aesculetin) were also detected [44]. In a study that investigated the content status of *S. hortensis* after the application of different extraction procedures (Soxhlet extraction, maceration, ultrasound-assisted extraction, microwave-assisted extraction, and subcritical water extraction), it was reported that the total phenolic content was between 119 and 151 mg/g, the total flavonoid content was between 5 and 28 mg/g, the condensed tannin content was between 41 and 73 mg/g, the gallotannin content was between 12 and 35 mg/g, and the total anthocyanin content was between 103 and 144 mg/g [45]. In many studies that investigated the pharmacological activity of *S. hortensis*, which has a large number of important compounds, it was reported to exhibit antioxidant [46], antimicrobial [47], and anticancer [44] activities. In addition, in many studies, many substances known for their antioxidant and cell-protective effects (such as anthocyanins, epigallocatechin-3-gallate, curcumin) were reported to improve lead-induced oxidative stress and prevent toxicity [48, 49, 50, 51]. Similarly, in this study, it was observed that the ethanolic extract of *S. hortensis*, which contains many substances with antioxidant activity, exhibited an antigenotoxic effect by providing a protective effect on reproductive performance and DNA damage in lead-treated *D. melanogaster*.

## CONCLUSION

In conclusion, it was determined in this study that reproductive performance decreased, and DNA damage increased in *D. melanogasters* given lead, whereas the application of ethanolic extract of *S. hortensis* plant reduced these negativities caused by lead. However, the absence of detailed antioxidant effects and pathway investigations represents a limitation. Lead and many heavy metals pose a risk both environmentally and in terms of human health. These results also necessitate the implementation of phytoremediation or bio-support strategies in contaminated areas. Considering the findings of the study, it is anticipated that determining the other pharmacological properties of *S. hortensis* and similar plants may play a role in developing new treatment methods against metal toxicity.

## DECLARATION

### Acknowledgement

Special thanks to the Assoc. Prof. Dr. Fahriye Zemheri-Navruz at Bartın University, Faculty of Science, Department of Molecular Biology and Genetics, for supporting this project.

### Authorship Contributions

Concept, Writing, Data Collection, and Interpretation: Gulin Donmez.

### Funding

The present research received no grant from any funding agency.

### Ethics approval and consent to participate

Ethics approval was not obtained for this study as it was the species we use is an invertebrate creature.

### Consent for publication

Not applicable.

### Competing interests

The authors declared that there is no conflict of interest.

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