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Genotoxic effects of oxyclozanide on hemocytes of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae

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Abstract: Oxyclozanide is a salicylanilide derivative anthelmintic drug with a well-known effect on parasites that cause infections in humans and animals. In this study, the effect of oxyclozanide on DNA damage in hemocytes of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae, which has been used as a model organism in many fields, was investigated. Hemolymph was collected from the last instar larvae (7th instar) reared on artificial diets containing oxyclozanide at different concentrations (0.003%, 0.03%, 0.3%, and 1.5%) under laboratory conditions and then hemocytes suspension was prepared. Genotoxic damage in hemocytes was determined by the comet assay which enables microscopically detecting DNA damage and is a very sensitive assay in chemical genotoxicity. When compared to the control group, tail length, tail DNA percent, and tail moment values were significantly increased parallel with increasing oxyclozanide concentrations. While the tail length was determined as 5.11 ± 0.46 µm in the control group, it was significantly increased in all tested groups to 13.17 ± 0.53, 27.98 ± 1.08, 98.44 ± 0.77, and 137.67 ± 0.74 µm, respectively. Similarly to tail length, tail DNA percentage and tail moment levels were also significantly increased from 12.86 ± 0.74 to 91.96 ± 0.31 at the highest concentration of oxyclozanide. These results showed that oxyclozanide caused DNA damage in the hemocytes of *G. mellonella*. It is also known that hemocytes are an important bioindicator in determining the genotoxicity of anthelmintics to be used as insecticides within environmentally friendly limits. It is thought that our results will contribute to the studies in this field.

Key words: *Galleria mellonella*, oxyclozanide, genotoxicity, hemocyte, anthelmintic

1. Introduction

Anthelmintic drugs are widely used in the treatment of parasitic diseases in humans and animals (Fissiha and Kinde, 2021). Oxyclozanide is a salicylanilide group anthelmintic drug to control and treatment of liver fluke *Fasciola hepatica* infection (fascioliasis) without adversely affecting the health of the host animals, and it disrupts oxidative phosphorylation in parasites inhibiting the malate dehydrogenase enzyme (Zhang et al., 2019).

The greater wax moth, *Galleria mellonella* has been widely used in infectious diseases, antimicrobial drugs, ecogenotoxicity, ecotoxicology, and stress physiology research area because of the short life cycle, easy maintenance, production of the large number of offspring, and absence of ethical concern (Mikulak et al., 2018; Pereira et al., 2018). Recent studies on genotoxicity assays in insects have opened up a broader perspective for DNA damage-related mechanistic studies, prescreening of chemicals as insecticides and also biomonitoring nontarget animals and environment (Mishraa et al., 2017). The comet assay is used to detect DNA damage caused by physical agents (gamma or UV lights) or xenobiotics (pesticides, insecticides, environmental pollutants) that have genotoxic or mutagenic effects in insect tissues as a sensitive and reliable method as other DNA sequence based assays (Augustyniak et al., 2016; Kaur et al., 2018). As known, hemocytes play important physiological roles such as immune, detoxification, antioxidant defence systems, and distribution of nutritive materials in the insects (Shen et al., 2011) thereby may be directly exposed to toxic substances circulating in the haemolymph leading to genotoxicity (Chatterjee and Walker, 2017; Singh et al., 2022). Shen et al., (2011) recorded the maximum value of tail DNA percent in hemocytes of the fourth instar larvae of silkworm *Bombyx mori* fed on mulberry leaves treated exposed to avermectin which is insecticide and anthelmintic showing positive relation between avermectin concentrations and DNA damage. DNA damage in hemocytes of destructive pest, melon fruit fly, *Zeugodacus cucurbitae* reared on diets containing different concentrations of a plant secondary

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metabolite allyl isothiocyanate was recorded by an increase in tail length (μm), tail DNA percent, tail moment and olive tail moment in coincided with decreased larval survivorship, deteriorated larval period, increased pupal and complete developmental times in each developmental stages (Singh et al., 2022).

There is increasing concern that our most published research findings are on the effects of mostly antibiotics (Büyükgüzel and Büyükgüzel, 2016; Hız et al., 2016; Keleş et al., 2021) and some anthelmintics on the biological traits, oxidative levels, and biochemical composition of insects (Kılıç et al., 2015; Sugeçti et al., 2016; Çalık et al., 2016; Sefer and Büyükgüzel, 2018; Sugeçti and Büyükgüzel, 2018; Büyükgüzel and Büyükgüzel, 2021; Keleş and Büyükgüzel, 2022). Toxic effects of oxyclozanide alone and in combination with other anthelmintics have also been shown on some mammalian systems (Wang et al., 2019; Kütahya et al., 2022). And also, there have been studies on the negative effects of salicylanilide anthelmintics, oxyclozanide (Çelik et al., 2019) and niclosamide on the biological traits, biochemical compositions, oxidative and antioxidative levels of *G. mellonella* (Büyükgüzel and Kayaoğlu, 2014) and *Drosophila melanogaster* (Ustundag et al., 2019), there have not yet any studies about the genotoxic effects of oxyclozanide and other anthelmintics in insect. However, there is no information about the genotoxic effects of this anthelmintic on insects. Therefore, this approach is the first time to evaluate levels of DNA damage in hemocytes after exposure to this anthelmintic. The aim of the present study is to determine the genotoxic effects of anthelmintic oxyclozanide on the hemocytes of the greater wax moth *G. mellonella* last instar larvae as a model organism to ascertain whether or not using anthelmintics as insecticides for pest insect management.

2. Material and methods

2.1. *G. mellonella* culture

Stock culture great wax moth *G. mellonella* L. (Lepidoptera: Pyralidae) was maintained by feeding newly hatched larvae on an artificial diet under laboratory conditions in an incubator (Nüve, FN 400) at 28 ± 2 °C, 65% ± 5% relative humidity, and continuous darkness. The artificial diet was composed of 420 g of bran, 150 mL of filtered honey, 150 mL of glycerol, 20 g of ground old dark honeycomb, and 30 mL of distilled water (Bronskil, 1961).

2.2. Feeding experiments

Oxyclozanide was purchased from Xi'an Quanao Biotech Co., Ltd. (Xian, Shaanxi, China). Oxyclozanide (2,3,5-trichloro-N-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxybenzamide, powder, 99%, 401.46 g/mol) was directly incorporated into the diet as g of 100 g of diet (%). The oxyclozanide concentrations used in the present study were based on our previous study investigating the effects of oxyclozanide on the survival, development, and total protein of *G. mellonella* (Çelik et al., 2019). Four different concentrations, namely 0.003%, 0.03%, 0.3%, and 1.5% were tested. An artificial diet without oxyclozanide was used as the control group. First instar larvae were reared on artificial diets containing oxyclozanide concentrations to last instar larvae (7th) for comet assays. The experiments were performed in four replicates with five larvae for each replicate.

2.3. Hemolymph collection

Hemolymph samples were collected according to Hyršl et al. 2008 and Büyükgüzel et al. 2010. The larvae were kept on ice for 5 min and surface sterilized in 95% ethanol. The hemolymph was collected into cold Eppendorf tubes by amputating the second pair of prolegs. Collected hemolymphs were centrifuged at 300 g, 4 °C, and 10 min for hemocyte separation. The obtained pellets were suspended by adding 150 μL of cold (Phosphate Buffer Saline (PBS)). A few crystals of phenylthiourea (PTU) were added to each sample to prevent melanization.

2.4. Comet assay

The comet assay was carried out by making modifications according to the protocol of Tice et al. (2000). Collected hemocytes were mixed with liquefied 0.65% agarose and transferred from this mixture to slides. The slides were then covered with 1% agarose and covered with coverslips. All slides were incubated at +4 °C for 30 min. The slides were separated from the coverslips and kept in a lysis solution containing with tris-borate-EDTA and sodium dodecyl sulfate (pH = 8.4) for 5 min. Slides were then run electrophoresis for 5 min. The samples were then washed with distilled water and stained with 80 μL of ethidium bromide (EtBr) (1:50, Sigma-Aldrich, St Louis, MO, USA). Images were examined with an Olympus DP72 digital camera mounted on a fluorescence microscope (Olympus BX53, 20x). Photographed comet image measurements were performed with the Kameram software (Kameram Gen3, version 3.5.1.0, Argenit Co., İstanbul, Turkey). To determine genotoxicity, the tail length (DNA migration length as μm), tail DNA % (the level of DNA migrated out of the nucleus expressed as the percent of DNA content in a cell), and tail moment (the tail length product and the total DNA fraction in the tail) parameters were used. Randomly selected 50 comet assays for each parameter were analyzed from oxyclozanide concentrations and the control group.

2.5. Statistical analysis

The data obtained with different concentrations of oxyclozanide were compared between each other and with the control group. IBM SPSS statistical software (2021) was used for the analyses. The data obtained from Comet assay were evaluated using a one-way analysis of variance

(ANOVA). The "LSD Test" was used to determine the significance of the difference between the mean values. The significance of the means was assessed at the 0.05 level of significance. Regression analysis was performed to test the correlation between oxyclozanide concentrations and each of the tail length, tail moment, and tail DNA%.

3. Results

Fluorescence microscopy comet images showing the genotoxic effect of oxyclozanide on the hemocytes of *Galleria mellonella* are given in Figure 1. These results showed that there were statistically significant differences between tested concentrations of the anthelmintic (0.003%, 0.03%, 0.3%, and 1.5%) and the control group (without oxyclozanide) for all comet parameters.

The tail length in the control group was found as 5.11 ± 0.46 µm. At 0.003%, 0.03%, 0.3%, and 1.5% concentrations of oxyclozanide significantly increased the tail lengths to 13.17 ± 0.53, 27.98 ± 1.08, 98.44 ± 0.77, and 137.67 ± 0.74 µm, respectively ($df = 4$, $F = 4506.427$, $p < 0.05$), (Figure 2).

The tail DNA% was significantly increased in a concentration-dependent manner ($df = 4$, $F = 1223.296$, $p < 0.05$). The low concentrations of oxyclozanide (0.003%, 0.03%, and 0.3%) caused a significant increase in the tail DNA% from a level of 12.86 ± 0.74 in the control group to 39.73 ± 0.84, 54.39 ± 1.32 and 89.03 ± 0.56, respectively. The tail DNA% was increased to 91.96 ± 0.3 at the highest oxyclozanide concentration (1.5%) by seven fold (Figure 3).

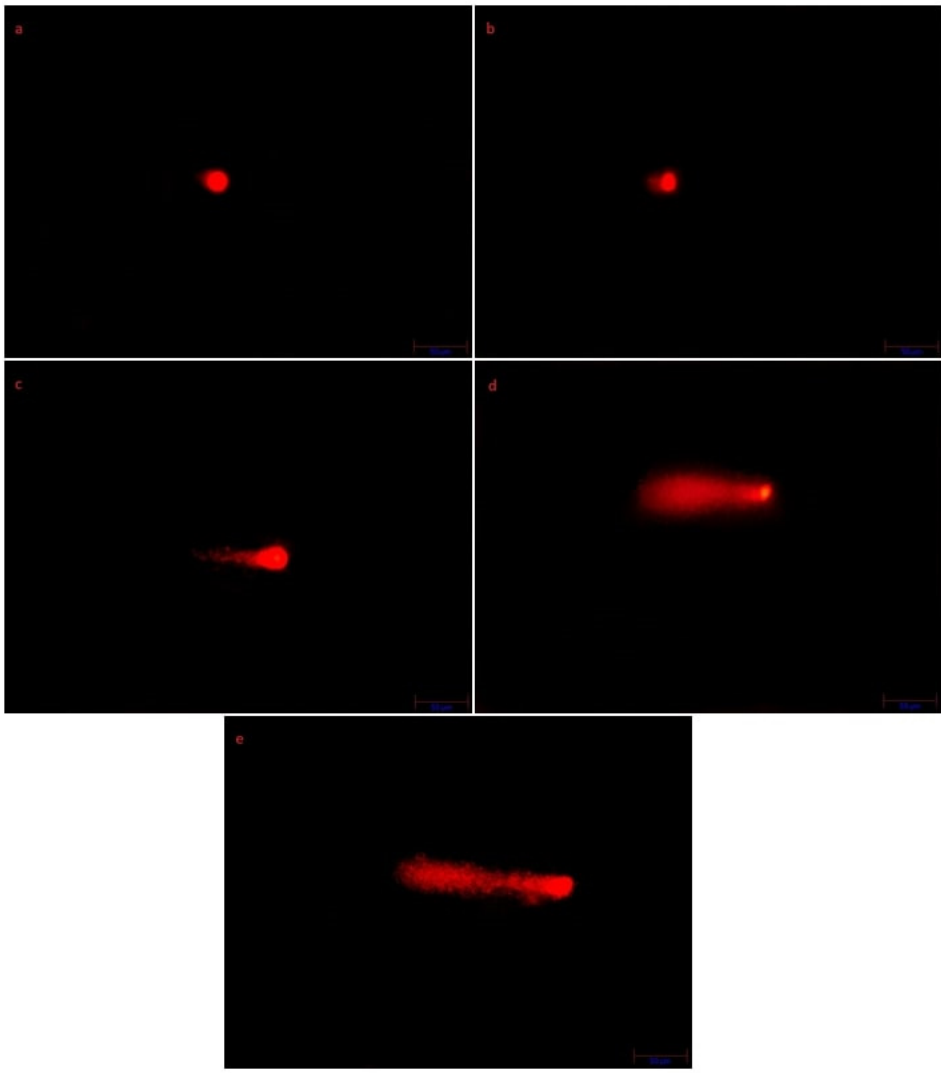

Figure 1. The comet assay images (20x) showing DNA damage in hemocytes of seventh instar larvae of *G. mellonella* reared on diet with oxyclozanide. (a) Control, (b) 0.003, (c) 0.03, (d) 0.3 g, and (e) 1.5% oxyclozanide concentration. The bar is 50 µm.

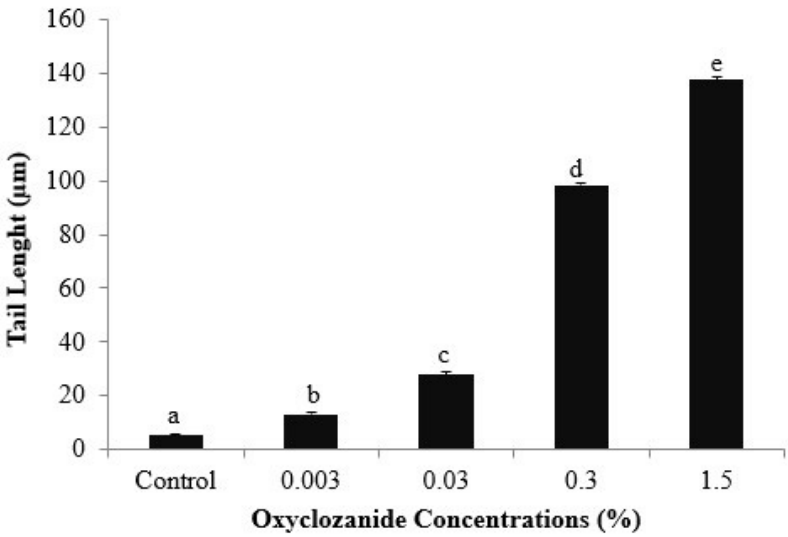

Figure 2. Tail length (µm) in hemocytes of seventh instar larvae of *G. mellonella*. Bars represent the means (± SE) of four replicates. Means followed by different letters are significantly different from each other, $p < 0.05$ (LSD Test).

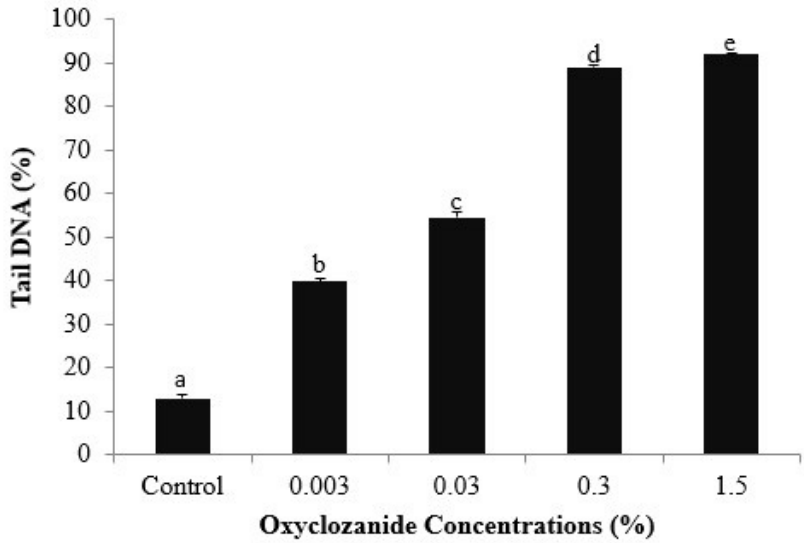

Figure 3. Tail DNA% in hemocytes of seventh instar larvae of *G. mellonella*. Bars represent the means (± SE) of four replicates. Means followed by different letters are significantly different from each other, $p < 0.05$ (LSD Test).

When all concentrations of oxyclozanide were in comparison to the control group, statistically significant differences were obtained in tail moment data as well as in tail length and tail DNA% results. Tail moment levels were recorded as 10.59 ± 0.30 (arbitrary unit) in the control group. At all tested concentrations of oxyclozanide (0.003%, 0.03%, 0.3%, and 1.5%), tail moment levels were determined as 13.53 ± 0.20, 21.53 ± 0.71, 55.19 ± 0.42, and 75.35 ± 0.38, respectively ($df = 4$, $F = 3158.537$, $p < 0.05$) (Figure 4).

Regression analysis of all the comet parameters assay showed a positive correlation between DNA damage parameters each other. Tail length was correlated with tail DNA% in insects ($R = 0.927$, $p = 0.023$). Tail length was correlated with a tail moment in insects ($R = 1$, $p < 0.001$). Tail DNA% was correlated with a tail moment in insects ($R = 0.922$, $p = 0.026$).

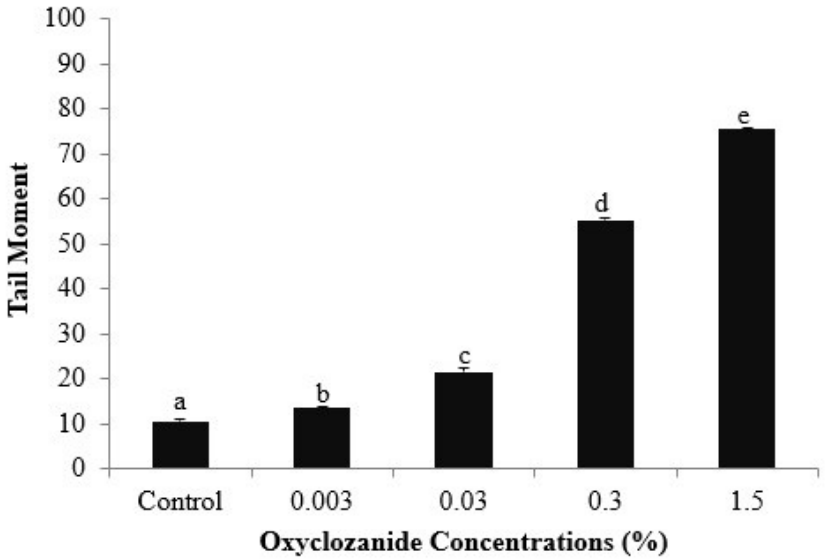

Figure 4. Tail moment in hemocytes of seventh instar larvae of *G. mellonella*. Bars represent the means (± SE) of four replicates. Means followed by different letters are significantly different from each other, $p < 0.05$ (LSD Test).

4. Discussion

DNA damage is of great importance in determining the genotoxicity of the chemicals on insects (Shen et al., 2011). The most commonly used parameters for genotoxicity are tail length, tail density, percentage of DNA in the tail, and tail moment (Singh et al., 2022). Our study demonstrated that oxyclozanide had genotoxic damage on larval hemocytes of *G. mellonella* in terms of significantly increased tail length, tail DNA percentage, and tail moment data in a concentration-dependent manner. The regression analysis also confirmed that there are significant and strong positive correlations between all DNA damage parameters each other. Similarly to our results, in a study indicated that organophosphate insecticide chlorpyrifos increased tail DNA percent, tail length, and tail moment in hemocytes of eri silkworms *Philosamia ricini* at the concentration of 2.0 mg/L and especially in tail length occurring ten-fold increase (Kalita et al., 2016). In another study supporting our results, it was expressed that heavy metal accumulation could play a role in causing DNA damage in hemocytes of grasshopper *Schistocerca gregaria* exposed to various doses of cadmium (Cd) and lead (Pb) (Yousef et al., 2010). In our results, it may be said that the increase in DNA damage in *G. mellonella* hemocytes depending on the amount of exposure to oxyclozanide may be caused by the consumption of oxyclozanide in insect hemocytes. Alaraby et al. (2019) inferred that Silver nanoparticles (AgNPs) significantly increase the percentage of tail DNA in hemocytes of *D. melanogaster* model insects and have a genotoxic effect by inducing reactive oxygen species (ROS). In our study, a genotoxic response may have occurred due to an increase in oxyclozanide-induced ROS. Our results coincided with the results of previous studies demonstrated that bioinsecticide, Azadirachtin (Duman and Altuntaş, 2018) and inorganic insecticide boric acid (Gwokyalya and Altuntaş, 2019) caused DNA damage resulting in increased tail density, tail moment and tail migration in hemocytes of model insect great wax moth *G. mellonella* in dose and time-dependent manner. Differences in levels of these genotoxic effects in different insects caused by different insecticides may be attributed to insect species, chemical structures, and types of insecticides and their concentrations. The present study demonstrated that a significant increase in all the DNA damage parameters including tail length, tail DNA percent, and tail moment was recorded in a dose-related manner, confirming that the consumption of oxyclozanide added to artificial diet caused significant genotoxicity in the hemocytes of seventh instar larvae of *G. mellonella*.

Foster and Downs (2005) indicated that before cellular apoptosis, DNA damage causes single-strand breaks. Therefore, as Porichha et al., (1998) indicated, tail formation or elongation is an indicator showing cell apoptosis and displays nuclear disruption or fragmentation in the form of DNA tails. Li et al., (2021) also reported avermectin which is an anthelmintic significantly increased DNA damage values of tail length and tail DNA percent and induced programmed cell death in the fall armyworm *Spodoptera frugiperda* cell line. It, therefore, is reasonable to suggest that oxyclozanide caused an increased level

of DNA damage in *G. mellonella* hemocytes resulting in nuclear fragmentation or disintegration in the form of DNA tails. Our comet images proved that the severity of the damage resulting in elongation occurs from the center to the outside in parallel to significantly increased tail DNA percent, tail length, and tail moment after exposure to high oxyclozanide concentrations. As Duman and Altuntaş (2018) suggested for azadirachtin insecticide on *G. mellonella*, induction in DNA damage parameters after consumption of an anthelmintic agent oxyclozanide in the present study may be a result of its impairment in DNA repair mechanism in hemocytes leading nuclear fragmentation or disintegration in the nucleus as DNA tails. Previous studies demonstrated that salicylanilide anthelmintic niclosamide and oxyclozanide significantly decreased the biological parameters such as survival rate, egg production, and hatching and extended the development of *G. mellonella* (Büyükgüzel and Kayaoğlu, 2014; Çelik et al., 2019), but their genotoxicity and the mode of the mechanism under this genotoxic effects and also their relation with deteriorated biological fitness of insects have just not been investigated. In this study, the genotoxic effect of oxyclozanide, an anthelmintic substance, on the larval hemocytes of *G. mellonella*, which is an important agricultural pest, was revealed. However, more detailed molecular studies are needed to elucidate the mechanism of DNA damage caused by oxyclozanide in the hemocytes of the insect *G. mellonella* larvae. Another output of this study is that results will give an insight into the future to develop new strategies and to sustain environmentally sound pest insect management.

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References

Alaraby M, Romero S, Hernández A, Marcos R (2019). Toxic and genotoxic effects of silver nanoparticles in Drosophila. Environmental and Molecular Mutagenesis 60 (3): 277-285. https://doi.org/10.1002/em.22262

Augustyniak M, Gladysz M, Dziewięcka M (2016). The comet assay in insects-status, prospects and benefits for science. Mutation Research/Reviews in Mutation Research 767: 67-76. https://doi.org/10.1016/j.mrrev.2015.09.001

Bronskill JF (1961). A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pyralidae). Journal of Lepidopterists' Society 15 (2): 102-104.

Büyükgüzel E, Büyükgüzel K (2016). Effect of acyclovir on the microbial contamination in the artifical and natural diets for rearing of *Galleria mellonella* L. larvae. Karaelmas Fen ve Mühendislik Dergisi 6 (1): 105-110.

Büyükgüzel E, Büyükgüzel K (2021). Oxidative impact of dietary triclabendazole in *Galleria mellonella*. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 27 (3): 301-306. https://doi.org/10.9775/kvfd.2020.25000

Büyükgüzel E, Kayaoğlu S (2014). The effect of niclosamide on some biological and physiological aspects of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Türkiye Entomoloji Dergisi 38 (1): 83-100. https://doi.org/10.16970/ted.87976

Chai WH, Li YR, Lin SH, Chao YH, Chen CH, Chan PC, Lin CH (2020). Antihelminthic niclosamide induces autophagy and delayed apoptosis in human non-small lung cancer Cells *in vitro* and *in vivo.* Anticancer Research 40 (3): 1405-1417. https://doi.org/10.21873/anticanres.14082

Chatterjee N, Walker GC (2017). Mechanisms of DNA damage, repair, and mutagenesis. Environmental and Molecular Mutagenesis 58 (5): 235-263. https://doi.org/10.1002/em.22087

Çalık G, Büyükgüzel K, Büyükgüzel E (2016). Reduced fitness in adults from larval, *Galleria mellonella* (Lepidoptera: Pyralidae) reared on media amended with the antihelmintic, mebendazole. Journal of Economic Entomology 109 (1): 182-187. https://doi.org/10.1093/jee/tov305

Çelik C, Büyükgüzel K, Büyükgüzel E (2019). The effects of oxyclozanide on survival, development and total protein of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Journal of the Entomological Research Society 21 (1): 95-108.

Duman E, Altuntaş H (2018). Genotoxicity of azadirachtin on *Galleria mellonella* L.(Lepidoptera: Pyralidae). Biodiversity and Conservation 11 (3): 24-30.

Fissiha W, Kinde MZ (2021). Anthelmintic Resistance and Its Mechanism: A Review. Infection and Drug Resistance 14: 5403-5410. https://doi.org/10.2147/IDR.S332378

Foster ER, Downs JA (2005). Histone H2A phosphorylation in DNA double strand break repair. The FEBS Journal 272: 3231-3240. https://doi.org/10.1111/j.1742-4658.2005.04741.x

Güven E, Pandir D, Baş H (2015). UV radiation-induced oxidative stress and DNA damage on Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae. Türkiye Entomoloji Dergisi 39 (1): 23-33. https://doi.org/10.16970/ted.06717

Gwokyalya R, Altuntaş H (2019). Boric acid-induced immunotoxicity and genotoxicity in model insect *Galleria mellonella* L. (Lepidoptera: Pyralidae). Archives of Insect Biochemistry and Physiology 101: e21588. https://doi.org/10.1002/arch.21588

Hasan MM, Todoriki S, Miyanoshita A, Imamura T, Hayashi T (2006). Soft-electron beam and gamma-radiation sensitivity and DNA damage in phosphine-resistant and-susceptible strains of *Rhyzopertha dominica*. Journal of Economic Entomology 99 (5): 1912-1919. https://doi.org/10.1093/jee/99.5.1912

Hız P, Erdem M, Büyükgüzel E, Büyükgüzel K (2016). The effect of gemifloxacin on some biological traits of *Galleria mellonella* (Lepidoptera: Pyralidae) Adults. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 22 (5): 777-784. https://doi.org/10.9775/kvfd.2016.15393

IBM SPSS Statistics (2021). User's manual, version 28.0. SPSS, Chicago, IL, USA.

Jung YY, Baek SH, Ha IJ, Ahn KS (2022). Regulation of apoptosis and autophagy by albendazole in human colon adenocarcinoma cells. Biochimie 198: 155-166. https://doi.org/10.1016/j.biochi.2022.04.014

Kalita MK, Haloi K, Devi D (2016). Larval exposure to chlorpyrifos affects nutritional physiology and induces genotoxicity in silkworm *Philosamia ricini* (Lepidoptera: Saturniidae). Frontiers in Physiology 7: 1-14. https://doi.org/10.3389/fphys.2016.00535

Kaur M, Chadha P, Kaur S, Kaur A, Kaur R, Yadav AK, Kaur R (2018). *Schizophyllum commune* induced genotoxic and cytotoxic effects in *Spodoptera litura*. Scientific Reports 8 (1): 4693. https://doi.org/10.1038/s41598-018-22919-0

Keleş V, Büyükgüzel E (2022). The effect of levamisole on some biological parameters of model organism *Galleria mellonella*. Karaelmas Fen ve Mühendislik Dergisi 12 (1): 93-100. https://doi.org/10.7212/karaelmasfen.1004103

Keleş V, Büyükgüzel K, Büyükgüzel E (2021). The effect of streptomycin on survival, development, and some biochemical aspects of *Drosophila melanogaster*. Turkish Journal of Zoology 45 (6): 432-441. https://doi.org/10.3906/zoo-2101-14.

Kılıç A, Büyükgüzel K, Büyükgüzel E (2015). The effect of anthelmintic triclabendazole on survival and development of *Galleria mellonella* (Lepidoptera: Pyralidae) L. reared on artificial diet. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 21 (6): 841-847. https://doi.org/10.9775/kvfd.2015.13731

Kütahya ZÖ, Kandır S, Avci Ç (2022). Effects of oxfendazole, oxyclozanide and oxfendazole-oxyclozanide combination on biochemical and hematological parameters in sheep and goats. Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi 7 (2): 108-115. https://doi.org/10.24880/maeuvfd.1081957

Li M, Zhang P, Xu W, Yuan J, Li Q, Tao L, Li Z, Zhang Y (2021). Avermectin induces toxic effects in insect nontarget cells involves DNA damage and its associated programmed cell death. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 249: 109130. https://doi.org/10.1016/j.cbpc.2021.109130

Mikulak E, Gliniewicz A, Przygodzka M, Solecka J (2018). *Galleria mellonella* L. as model organism used in biomedical and other studies. Epidemiological Review 72 (1): 57-73.

Mishraa N, Srivastavaa R, Agrawalb UR, Tewaria RR (2017). Review an insight into the genotoxicity assessment studies in dipterans. *Mutation Research/Reviews in Mutation Research* 773: 220-229. https://doi.org/10.1016/j.mrrev.2016.10.001

Pereira TC, De Barros PP, Fugisaki LRDO, Rossoni RD, Ribeiro FDC, De Menezes RT, Junqueira JC, Scorzoni L (2018). Recent advances in the use of *Galleria mellonella* model to study immune responses against human pathogens. Journal of Fungus 4 (4): 128. https://doi.org/10.3390/jof4040128

Porichha SK, Sarangi PK, Prasad R (1998). Genotoxic effect of chlorpyrifosin *Channa punctatus*. Cytology and Genetics 9: 631-638.

Sefer EN, Büyükgüzel K (2018). The Effect of piperazine on survivorship and development of *Galleria mellonella*. Karaelmas Fen ve Mühendislik Dergisi 8 (1): 365-372. https://doi.org/10.7212%2Fzkufbd.v8i1.1248

Shen W, Zhao X, Wang Q, Niu B, Liu Y, He L, Weng H, Meng Z, Chen Y (2011). Genotoxicity evaluation of low doses of avermectin to hemocytes of silkworm (*Bombyx mori*) and response of gene expression to DNA damage. Pesticide Biochemistry and Physiology 101: 159-164. https://doi.org/10.1016/j.pestbp.2011.08.011

Singh S, Diksha Mahajan E, Sohal SK (2022). Appraisal of growth inhibitory, biochemical and genotoxic effects of allyl isothiocyanate on different developmental stages of *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae). Scientific Reports 12: 10363-10375. https://doi.org/10.1038/s41598-022-14593-0

Sugeçti S, Büyükgüzel E, Büyükgüzel K (2016). Laboratory assays of the effects of oxfendazole on biological parameters of *Galleria mellonella* (Lepidoptera: Pyralidae). Entomological Science 51 (2): 129-137. https://doi.org/10.18474/JES15-36.1

Tice RR, Agurell E, Anderson D, Burlison B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environmental and Molecular Mutagenesis 35 (3): 206-221. https://doi.org/10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-J

Ustundag G, Buyukguzel E, Buyukguzel K (2019). The effect of niclosamide on certain biological and biochemical properties of *Drosophila melanogaster*. European Journal of Biology 78 (1): 29-38. https://doi.org/10.26650/EurJBiol.2019.0003

Wang W, Dong Z, Zhang J, Zhou X, Wei X, Cheng F, Li B, Zhang J (2019). Acute and subacute toxicity assessment of oxyclozanide in Wistar rats. Frontiers in Veterinary Science 6: 294. https://doi.org/10.3389/fvets.2019.00294

Yousef HA, Afify A, Hasan HM, Meguid AA (2010). DNA damage in hemocytes of *Schistocerca gregaria* (Orthoptera: Acrididae) exposed to contaminated food with cadmium and lead. Natural Sciences 2 (4): 292-297. https://doi.org/10.4236/ns.2010.24037

Zhang J, Bai Y, Li B, Zhou X, Si H, Zhang J (2019). Determination and pharmacokinetics study of oxyclozanide suspension in cattle by LC-MS/MS. BMC Veterinary Research 15: 210. https://doi.org/10.1186/s12917-019-1963-0