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The Effect of Bellis on Levels of Plasma Paraoxonase Activity, High-Density Lipoprotein and Malondialdehyde in *Cyprinus carpio L.*, 1758

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Abstract: The aim of this study is to investigate the effect of Bellis, which is used as a fungicide, on the levels of plasma paraoxonase activity (PON), high-density lipoprotein (HDL), and malondialdehyde (MDA) in *Cyprinus carpio*. A total of 24 (200-300 g) *C. carpio* fish including eight in each group were used. Fish were divided into three groups; Group I (control), Group II (0.025 mg/L Bellis), and Group III (0.050 mg/L Bellis). Blood samples were collected from the fish after 14-day application and their plasmas were separated. Plasma samples were analyzed for PON activity, HDL, and MDA levels. The study findings revealed that PON activity and HDL levels were significantly higher ($p<0.001$) in Group I, compared to Group II and Group III; whereas, MDA levels were lower ($p<0.05$). When the dosage of fungicide increased, PON activity and HDL levels dropped but MDA levels increased. In according to these findings, it was concluded that Bellis, which has been widely used in recent years, may trigger oxidative stress in fish depending on the increasing dose.

Keywords: bellis, *Cyprinus carpio*, malondialdehyde, oxidative stress, paraoxonase activity

Cyprinus carpio L., 1758'de Bellis'in Plazma Paraoksonaz Aktivitesi, Yüksek Dansiteli Lipoprotein ve Malondialdehit Düzeyleri Üzerine Etkisi

Özet: Bu çalışmada, fungusit olarak kullanılan Bellis'in *Cyprinus carpio'*da plazma paraoksonaz aktivitesi (PON), yüksek dansiteli lipoprotein (HDL) ve malondialdehit (MDA) düzeylerine etkisinin araştırılması amaçlandı. Her grupta 8 adet olmak üzere toplam 24 adet (200-300 g) *C. carpio* kullanıldı. Balıklar Grup I (kontrol), Grup II (0,025 mg/L Bellis) ve Grup III (0,050 mgl/L Bellis) olmak üzere üç gruba ayrıldı. 14 günlük uygulamanın ardından balıklardan kan örneği alındı ve plazmaları ayrıldı. Elde edilen plazma örneklerinde PON aktivitesi, HDL ve MDA düzeyleri çalışıldı. Çalışmada elde edilen bulgulara göre Grup I'de PON aktivitesi ve HDL düzeyleri Grup II ve Grup III'e göre anlamlı şekilde ($p<0,001$) daha yüksek bulunurken, MDA düzeyleri ise daha düşük bulundu ($p<0,05$). Fungisit uygulanan balıklarda artan doza bağlı olarak PON aktivitesi ve HDL düzeyleri azalırken MDA düzeylerinin ise arttığı tespit edildi. Bu sonuçlara göre son yıllarda yaygın bir şekilde kullanılan Bellis'in artan doza bağlı olarak balılarda oksidatif stresi tetikleyebileceği sonucuna varıldı.
Anahtar kelimeler: bellis, *Cyprinus carpio*, malondialdehit, oksidatif stres, paraoksonaz aktivite

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INTRODUCTION

Since the twentieth century, the natural environment and human health have begun to be adversely affected as a result of excessive application of pesticides. Pesticides used to control agricultural pests can persist in the soil for an extended time period without decomposing and cause detrimental effects on other living beings in the environment (Abdollohi et al., 2004; Deveci et al., 2017; Dogan et al., 2021). These pesticides, which do not decompose in the soil, migrate through the food chain and accumulate in a variety of living beings, resulting in bioconcentration. As they migrate through the food chain, these accumulations reach a higher concentration at each stage. Pesticides and breakdown products that penetrate the body through the food chain eventually have negative impacts on tissues and organs in the course of time (Deveci et al., 2015; Strungaru et al., 2019; Kayhan, 2020).

Fungicides are a type of pesticide that is frequently used in agricultural lands to control pathogenic fungi on plants. Fungicides are used to treat and preserve maize, wheat, olive, pistachio, and fruit, as well as other plants (Chen et al., 2008; Avenot et al., 2008; Temiz, 2019). Bellis® is one of the pesticides that have been increasingly used in recent years in Turkey, particularly in and around Gaziantep, to control pistachio, olive, and pomegranate pests. Bellis® contains anilide and strobilurin (25.2% Boscalid + 12.8% Pyraclostrobin, WG). Boscalid is a novel broad-spectrum fungicide that belongs to the anilide family of fungicides. Its action mode and range are different from those of strobilurins and most the other fungicides. Boscalid inhibits complex II in the mitochondrial electron transport chain; whereas, pyraclostrobin inhibits the complex III (Avenot et al., 2008; Lagunas-Allué et al., 2015; Ozkılınc and Kurt, 2017; Aksakal, 2020).

Fish are used as indicators to identify pollution in the aquatic ecosystem (pesticides, heavy metals, and other chemicals) and its impact on other living beings (Van der Oost et al., 2003; Temiz, 2019). Many environmental factors increase the number of oxidant molecules in living beings by stimulating reactive oxygen species (ROT) (Li et al., 2011; Deveci et al., 2021). Lipid peroxidation, which occurs as the result of pesticide toxicity, causes cellular damage to cells by affecting their antioxidant system. Malondialdehyde (MDA), a product of lipid peroxidation, is a significant indicator of oxidative stress (Kehrer, 1993). Cells activate their antioxidant defense mechanisms to neutralize the harmful effects of oxidative stress induced by oxidant molecules. Catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST), and glutathione peroxidase (GSH-Px) are the primary defensive mechanisms in fish (Kayhan, 2020). Paraoxonase enzyme (PON), a calcium-dependent esterase enzyme synthesized in the liver, is transported in the serum bound to high-density lipoproteins (HDL). PON acts as an antioxidant by protecting low-density lipoprotein (LDL) from oxidation and neutralizing hydrogen peroxide and other free radicals (Costa et al., 2003; Deveci and Karapehlivan, 2018). PON has been extensively evaluated in toxicological studies in recent years due to its ability to hydrolyze organophosphate pesticides and nerve gases.

The aim of this study is to investigate the effects of Bellis, an important fungicide that has been increasingly used in recent years, on the plasma PON activity, HDL, and MDA levels in *C. carpio.*

MATERIALS and METHODS

In this study, a total of 24 *C. carpio* weighing 200-300 grams that were caught in the İslahiye Tahtaköprü Dam Lake were used. The fish were taken to the laboratory and they were kept in polyethylene tanks for five days to adapt to their new habitat. They were separated into three groups including eight fish in each group following their adaptation to the new habitat. In the control group, Group I, normal water was supplied to the fish’s tanks without adding any substance. The fish in

Group II were kept in tanks treated with 0.025 mg/L Bellis® (25.2 % Boscalid + 12.8 % Pyraclostrobin, BASF TURK), whereas the fish in Group III were kept in tanks treated with Bellis® at a dosage of 0.050 mg/L. Given the absorption and evaporation factors of the fungus by the fish, water was changed daily and new dosages were introduced (APHA, 1981). At the end of the fourteenth day, blood samples were drawn from the caudal vein of the fish. Plasma was obtained from blood samples by centrifuging them at 3000 rpm for 10 minutes and kept at -20 °C until analysis began. Plasma PON activity was measured spectrophotometrically according to the method by Eckerson et al., (1983). MDA levels were measured colorimetrically in the spectrophotometer according to the methods described by Yoshoiko et al., (1979). Commercial kits were used to measure HDL levels in an autoanalyzer.

Statistical Analysis

The SPSS 21.0 package software was used to conduct a statistical analysis of the data. The one-way analysis of variance (One Way ANOVA) was employed to determine if there were differences between the groups in terms of PON, HDL, and MDA. Post-hoc (Tukey LSD) analysis was conducted to determine which groups had a significant difference. The results are expressed as mean ± standard deviation ($\bar{x}$±SD). The value of $p<0.05$ was considered statistically significant.

FINDINGS

Table 1 shows the plasma analysis results from fish (Groups II and III) and control (Group I) after 0.025 and 0.05 mg/L Bellis fungicide was added to their tanks for the experimental study, as well as the changes in their parameters.

Table 1. Comparison of PON, HDL, and MDA levels in *C. carpio* with and without Bellis.

Parameters	Group I (Control) mean±SD	Group II (0.025 mg/L) mean±SD	Group III (0.050 mg/L) mean±SD	p
PON (U/L)	29.86±3.66[a]	20.07±2.36[b]	18.55±1.96[b]	<0.001
HDL (mg/dL)	36.42±2.44[a]	29.57±2.44[b]	26.85±2.99[b]	<0.001
MDA (µmol/L)	1.47±0.13[b]	1.74±0.25[a]	1.83±0.24[a]	<0.05

[a, b] The values denoted by different letters on the same line are statistically different. SD: Standard deviation.

When plasma samples from Group I fish that was not been treated were compared with plasma samples from Group II and Group III fish that was treated with, respectively, 0.025 mg/L and h 0.050 mg/L fungicide, the PON activity, and HDL levels were significantly higher ($p<0.001$). When the MDA levels of the control group and the other groups (Group II and Group III) were compared, it was determined that the control group had a statistically significantly lower MDA level ($p<0.05$) (Fig. 1).

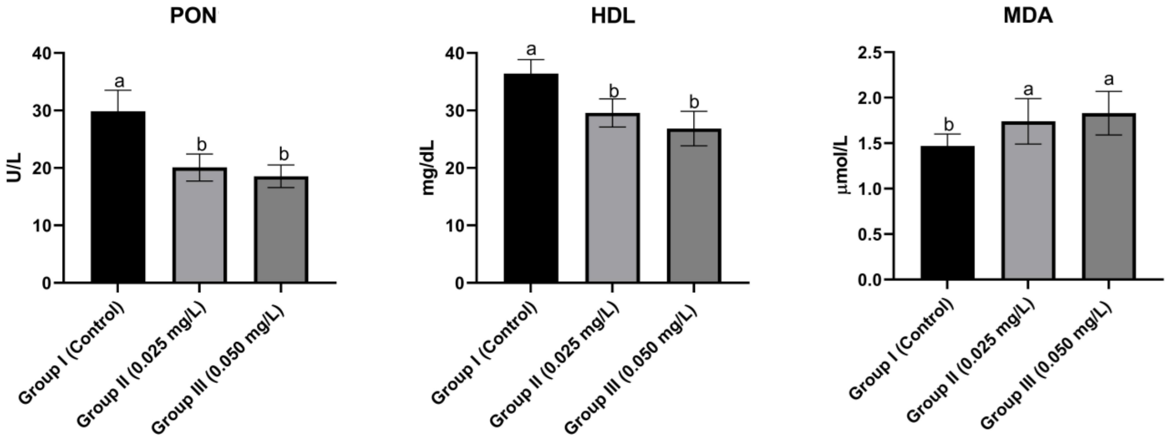

Figure 1. Schematic representation of the PON, HDL, and MDA levels of the groups. [a, b] The values indicated with different letters between different groups are statistically different.

DISCUSSION and CONCLUSION

In parallel to agricultural advancements, the use of pesticides has become significantly widespread to increase both the quantity and quality of the product. Along with being extremely effective in agricultural pest control, pesticides have a detrimental effect on ecosystem health as a result of their long-term and unconscious usage (Çelik et al., 2020; Kaya et al., 2012). Particularly, the contamination of groundwater and other water resources with pesticides poses a potential threat to lifeforms in the aquatic ecosystem. Fish have been extensively employed in recent years to determine the toxicity of the aquatic ecosystem and to predict the possible impacts of agricultural chemicals on other creatures (Selvi et al., 2004; Kaya et al., 2014; Deveci et al., 2017).

Determining the effects of Bellis, which is used intensively in the control of various pests in agricultural lands, on PON, HDL, and MDA levels in fish is important in terms of using pesticides at the appropriate level and minimizing environmental pollution. Additionally, determining the effects of this fungicide on fish enables the prediction of metabolic alterations that may develop in other organisms that ingest these fish. Despite our comprehensive literature review, we have not encountered any study examining the effects of Bellis on PON activity, HDL and MDA levels, this study has an original quality to provide data for other studies. In this regard, this study will serve as a valuable reference for future studies.

Some studies reported that pesticide treatments in fish induced oxidative stress in fish by increasing reactive oxygen species (ROS) in their cells and tissues (Yonar and Sakin, 2011; Deveci et al., 2017; Nur and Deveci, 2018). Oxidative stress negatively affects life by causing genotoxic effects, lipid peroxidation and enzyme inhibitions. Lipid peroxidation, which occurs as a result of the toxic effects of pesticides, is an important indicator of oxidative stress and can be demonstrated by measuring MDA levels (Fırat and Aytekin, 2018; Toroser et al., 2007).

In a study using boscalid, the active component in Bellis, it was reported that while there was an increase in MDA levels depending on the increasing dose of active substance, there was a decrease in the levels of antioxidant enzymes SOD and GST and a significant increase in the CAT level at high doses (Aksakal, 2020). In their study in which they examined the toxic effect of boscalid on zebrafish, Zang et al., (2017) reported that GPx, SOD, CAT, and MDA levels changed based on the boscalid concentration and exposure time. According to the same study, the increase in antioxidant enzyme activity on the seventh day was a reaction to oxidative stress, and the level of MDA increased significantly after the 21-day treatment. Similarly, the studies utilizing different pesticides reported that MDA, a product of lipid peroxidation, increased in fish blood and different tissues (Durmaz et al., 2006; Isik and Celik, 2008; Deveci et al., 2016; Dogan et al., 2021). MDA levels increased in fish treated with different dosages of Bellis fungicide in the present study, similar to the fish studies conducted with different pesticides above.

The antioxidant properties of the PON enzyme, which is synthesized in the liver and transported depending on HDL, are due to its ability to protect HDL and LDL from oxidation. However, the PON enzyme was reported to be sensitive to oxidative stress and to be subjected to activity loss depending on the increasing oxidant levels (Costa 2003; Deveci et al., 2015; Deveci and Karapehlivan, 2018). A study reported that in trout exposed to different dosages of the organophosphate pesticide glyphosate, the total oxidant level rose depending on dose increase but the total antioxidant level and PON activity decreased (Nur and Deveci, 2018). In their study, Atamanalp et al., (2021) also administered N-acetyl cysteine, an antioxidant, to the fish to which pesticides were applied. They reported that pesticide exposure increased the activities of SOD, CAT, GPx, and PON in all tissues in the group to which the N-acetyl cysteine was administered (Atamanalp et al., 2021). Similar to other studies, the present study revealed a decrease in PON activity and HDL levels in fish treated with different dosages of Bellis fungicide. We think that the decrease in PON activity and HDL levels can be associated with oxidative stress induced by lipid peroxidation of Bellis fungicide.

In conclusion, in the light of the data of the present study, it is considered that Bellis may induce oxidative stress in aquatic organisms, hence causing metabolic alterations in other creatures that ingest these organisms.

Conflict of interest: There is no conflict of interest among the authors.

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