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The effect of the pesticide Klaxon on the oxidative stress and the antioxidant responses of the Zebra mussel (*Dreissena polymorpha*)

El efecto del pesticida Klaxon sobre el estrés oxidativo y las respuestas antioxidantes del mejillón cebra (*Dreissena polymorpha*)

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ABSTRACT

Klaxon pesticide represents a novel generation of insecticide employed in the control of diseases, pests and weeds in select agricultural regions. Pesticides that enter the aquatic environment indirectly have a detrimental impact on the organisms that inhabit this environment, and humans are ultimately exposed to these chemicals through the food chain. The present study investigated the toxicity of the klaxon pesticide in *Dreissena polymorpha*, a suitable bioindicator of water pollution, through the analysis of oxidative stress and metabolic biomarkers. The effects of klaxon at concentrations of 0.01, 0.15 and 0.30 mg·L^{-1} on oxidative stress and antioxidant changes in *D. polymorpha* were determined over a 24 – and 96–hour period. Enzyme–linked immunosorbent assay (ELISA) kits were employed to quantify the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and thiobarbituric acid (TBARS), as well as to assess the levels of reduced glutathione (GSH). ELISA kits were used to determine results. Statistical evaluation of biomarker analyzes was performed using the SPSS 24.0 package program one–way ANOVA (Duncan 0.05) test. Significant decreases in GSH levels (P<0.05); significant increases in TBARS levels (P<0.05) were observed. Significant decreases (P<0.05) in SOD, CAT and GPx activities were observed. Considering the study data, it was determined that the klaxon pesticide penetrating the body of the living organism caused oxidative stress and changes in enzyme activities in *D. polymorpha*.

Key words: Klaxon; *Dreissena polymorpha;* oxidative stress; antioxidant response

RESUMEN

El pesticida Klaxon representa una nueva generación de insecticidas empleados en el control de enfermedades, plagas y malezas en regiones agrícolas seleccionadas. Los pesticidas que ingresan al ambiente acuático indirectamente tienen un impacto perjudicial en los organismos que habitan este ambiente, y los humanos están finalmente expuestos a estos químicos a través de la cadena alimentaria. El presente estudio investigó la toxicidad del pesticida Klaxon en *Dreissena polymorpha*, un bioindicador adecuado de la contaminación del agua, a través del análisis del estrés oxidativo y biomarcadores metabólicos. Se determinaron los efectos del klaxon en concentraciones de 0,01; 0,15 y 0,30 mg·L^{-1} sobre el estrés oxidativo y los cambios antioxidantes en *D. polymorpha* durante un período de 24 y 96 horas. Se emplearon kits de ensayo inmunoabsorbente ligado a enzimas (ELISA) para cuantificar las actividades de superóxido dismutasa (SOD), glutatión peroxidasa (GPx), catalasa (CAT) y ácido tiobarbitúrico (TBARS), así como para evaluar los niveles de glutatión reducido (GSH). Se utilizaron kits ELISA para determinar los resultados. La evaluación estadística de los análisis de biomarcadores se realizó utilizando la prueba ANOVA unidireccional (Duncan 0,05) del paquete SPSS 24.0. Se observaron disminuciones significativas en los niveles de GSH (P<0,05); aumentos significativos en los niveles de TBARS (P<0,05). Se observaron disminuciones significativas (P<0,05) en las actividades de SOD, CAT y GPx. Considerando los datos del estudio, se determinó que el pesticida klaxon que penetró en el cuerpo del organismo vivo causó estrés oxidativo y cambios en las actividades enzimáticas en *D. polymorpha*.

Palabras clave: Klaxon; *Dreissena polymorpha*; estrés oxidativo; respuesta antioxidante

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INTRODUCTION

Environment is the physical, chemical, biological, social, economic and cultural context in which humans and other living things relate and interact throughout their lives. Pollution affects the entire ecosystem, including humans, and occurs mainly in nature in the form of air, soil and water pollution. The processes of industrialisation, technological development and population growth are having a detrimental impact on the natural environment, with pollution levels rising at an alarming rate. In both urban and rural environments, contamination of air, soil and water—natural assets—arises for a variety of reasons, affecting the survival of flora and fauna and, through the food chain, human health [1].

Pesticides are defined as chemicals used during the production, storage and consumption of agricultural products in order to destroy insects, animals, microorganisms, weeds and other harmful organisms that damage agricultural products or to reduce that damage or have the potential to damage agricultural products [2, 3]. Persistent pesticides, although they have a low concentration in the water matrix, are more dangerous due to their high stability and bioaccumulation properties. The main reason for high bioaccumulation in aquatic organisms is the high solubility of certain pesticide groups in water [4]. Klaxon 20 SC is a new generation insecticide used against diseases, pests and weeds in integrated control programs with 200 $g \cdot L^{-1}$ Chlorantraniliprole as active ingredient. It is used for harmful organisms such as *Cydia pomonella*, *Leucoptera scitella*, *Lobesia botrana*, *Anarsia lineatella*, *Cydia molesta*, *Agrotis ipsilon*, *Agrotis segetum*, *Sesamia spp.*, *Ostrinia nubilalis*, *Earias insulana*, *Spodoptera littoralis* and *Helicoverpa armigera* [5]. Concurrent with the rapid growth of the global population, concerns about food security and malnutrition have emerged as significant challenges, particularly in developing countries. This situation has brought along the necessity of using agricultural areas in the most efficient way. In addition to the use of new technologies in agriculture, another way to increase yield is to protect plants from all kinds of pests that prevent the development of plants and reduce their yield by using pesticides. For this purpose, pesticides are widely used for preventive purposes in the fight against pests in agricultural areas. The fact that pesticides have high efficacy against harmful organisms, give fast results, protect the product from toxin–secreting organisms and are economical when used consciously and in a controlled manner causes them to be widely used [6].

After pesticide practices, the pesticide mixtures used do not remain in the plant, but mix into the soil and air. The spread of pesticides in the aquatic ecosystem varies depending on the environmental conditions, physical, chemical and formulation structure of the pesticide. In addition, soil type, slope, vegetation cover and rainfall also play a role in the contamination of water by pesticides. This contamination can have an acute or chronic toxic effect on aquatic organisms, negatively affecting their reproductive abilities and causing a decrease in their populations [7]. Pesticides, which degrade very slowly due to their molecular structure, can reach harmful and even toxic values through biological and physical accumulation even if they diluted to low concentrations in the aquatic environment. Pesticides in water accumulate in the fat tissues of living organisms and their concentrations always increase when they pass to fish and birds feeding on them through the food chain [8]. Even trace amounts of pesticide residues in water can prevent the development of zooplankton and phytoplankton, which are very important in the food chain of aquatic organisms [9].

Dreissena polymorpha is one of the most important invasive species living in freshwater. They are both economically and ecologically damaging as they cause corrosion, clog water filters, restrict the lives of other living organisms, block water flow and cause corrosion with the community they form in their environment. However, the life tolerance of zebra mussels is quite high. They can adapt to very low and very high temperatures, prolonged starvation, different levels of dissolved oxygen and calcium [10, 11]. The widespread and reliable use of these creatures in toxicological studies can be attributed to their long life span, limited movement and filter feeding. In addition, although it is known as an invasive species, it is also suitable as a biological monitoring and model species and is easily used in the investigation of anthropoenic stress effects in aquatic environments [11, 12, 13].

This study will be an original study to determine the changes in TBARS and GSH levels and SOD, CAT and GPx enzyme activities of zebra mussels (*Dreissena polymorpha*) in Keban Dam Lake (Elazığ) due to the use of the pesticide klaxon in agricultural spraying. It is thought that the findings of the research will direct on the impact of agricultural activities, which are becoming more and more widespread around our reservoir, and will make an important contribution to future planning.

MATERIALS AND METHODS

Test organism

Zebra mussel (*Dreissena polymorpha*) is a reference species for ecotoxicological studies in aquatic ecosystems [14]. These mussels are mainly distributed in lakes and reservoirs in Turkey. As a species that is not endangered and can encountered continuously in nature, has stable behavior and sufficient body size, it is easier to sample than other species [15, 16, 17] and has been preferred because it is not selective in food intake [16]. *D. polymorpha* was collected by hand from the cages of an aquaculture company in Keban Dam Lake and brought to Munzur University Faculty of Fisheries in plastic box.

Adaptation of test organisms to the laboratory environment

D. polymorpha were brought to the laboratory alive and placed in 80×40×25 cm aquariums. A photoperiod (12 hours (h) of light and 12 h of dark illumination) was applied. Ambient temperature was maintained at 18°C. Cultured plankton (*Chlorella vulgaris + Navicula cryptocephala*) was given as *D. polymorpha* food. An external filter and an air motor were used to ensure adequate oxygen supply in the aquaria. During this adaptation process, the health status of the organisms was observed and noted. Metric–meristic measurements of the species were taken before starting the experimental study. Organisms with similar size (W:1.01 ± 0.25 g, L:20.34 ± 2.04 mm, h:9.80 ± 1.08 mm, w:10.10 ± 1.01 mm) and characteristics were used to avoid misleading experimental results. Healthy individuals that reacted to light and sound by closing their shells were selected.

Chemical

Klaxon 20 SC active ingredient pesticide used in the study was purchased from Agrofarm Kimya Comp. registered trademark

Suspension Concentrate (SC) 200 g·L^{-1} Chlorantraniliprole was purchased.

Determination of sublethal concentrations

To determine non–lethal concentrations, a comparison of the concentrations obtained in practice with the values of the rates of diffusion into the environment was taken into account.

Research design

Five healthy models of the same size were placed in separate 30 L glass tanks. Air motors were installed to meet their oxygen needs. For the experimental study, 4 groups were formed with 1 control group (14 individuals were placed in each group, including replications). Set to two time periods (24 or 96 h) in all four groups. Acute toxicity tests were not performed considering the environmental effects of xenobiotic substances such as pesticides. Sublethal concentrations were determined by scanning the literature for experimental groups and considering the concentration values used in toxicological studies [18].

- Group A (Control); practice group: group without any klaxon.
- Group B; was exposed to klaxon concentrations of 0.01 mg·L^{-1} for 24 and 96 h.
- Group C; was exposed to klaxon concentrations of 0.15 mg·L^{-1} for 24 and 96 h,
- Group D; was exposed to klaxon concentrations of 0.30 mg·L^{-1} for 24 and 96 h.

All stages in the experimental practices were performed in three repetitions.

Biochemical evaluation

Three test organisms were randomly selected from the aquaria and separated with a scalpel. The organisms were subjected to cold shock treatment (ice water for 30 min), 0.5 g of sample was taken from each organism to evaluate the antioxidant properties, homogenized (Daihan brand, Hg-15D Digital ultraturax model, Korea) and 1/5 w/v phosphate buffered saline solution (PBS buffer) was added. After homogenization, the samples were centrifuged (NUVE brand, NF1200R model centrifugal, Turkiye) at 17,000 rpm in a refrigerated centrifuge for 15 min, and the supernatants were stored in the deep freezer (Daihan brand, Wisd ultra freezer model, Korea) at -86°C until measurement was taken.

In this study, GSH and TBARS levels as well as SOD, CAT and GPx activities were measured using an ELISA kit (Agilent brand, BioTek 800 TS Absorbance Reader, USA) for the biochemical response of *D. polymorha* mussel individuals exposed to klaxon pesticide. TBARS (Catalog No. 10009055), GSH (Catalog No. 703002), SOD (Catalog No. 706002), CAT (Catalog No. 707002) and GPx (Catalog No. 703102) levels in tissues were found with the kits bought from CAYMAN Chemical Company.

Statistical analysis

Statistical evaluation of biomarker analyzes was performed using the SPSS 24.0 package program one–way ANOVA (Duncan 0.05) test.

RESULTS AND DISCUSSION

Aquatic ecosystems can be contaminated with pesticides through a variety of means, including overflows, agricultural runoff, spray drifts, wastewater discharge, and seepage. Due to the accumulation of toxic substances in various tissues and organs of aquatic organisms, the concentration of pesticides in aquatic organisms is several times higher than in the aquatic ecosystem [19]. Aquatic ecosystems are adversely affected by high levels of pesticide residues in water and sediments. This leads to significant loss of biodiversity [20].

TBARS level

The TBARS level increased with increasing concentration compared to the control (A). While groups C and D showed a statistically significant (P<0.05) increase in both 24 and 96-h practice groups compared to the control, the increase in group B was found statistically insignificant (P>0.05) (FIG. 1).

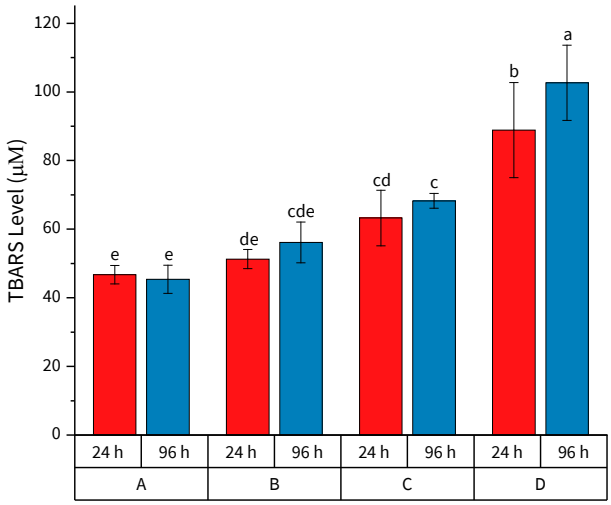

FIGURE 1. TBARS (µM) levels of *Dreissena polymorpha* exposed to different concentration of klaxon. Different letters on the bars indicate a statistically significant difference between the groups within same treatment period (P<0.05)

Lipid peroxidation, which affects polyunsaturated fatty acids (in membrane phospholipids), is a degenerative process that results in the formation of toxic aldehydes. These react with protein and non–protein substances to cause widespread varies in cell membranes [21]. If antioxidant defences are insufficient to neutralise excess ROS that may be generated during biotransformation, end product of lipid peroxidation, MDA (malondialdehyde) can ocur [22]. TBARSs are used to survey lipid peroxidation produce body fluids, in cells and tissues [23].

A review of the literature reveals that various pesticide species have been shown to induce alterations in TBARS levels in aquatic organisms, thereby corroborating the findings of this study. Ji *et al.* [24] reported increases in TBARS levels in *Carassius auratus* as a result of medium–time exposure to Benzo(k)fluoranthene (BkF) alone and together PCB118 and dichlorodiphenyltrichloroethane (DDT). Marins *et al.* [25] observed increases in TBARS levels in *Rhamdia quelen* species exposed to imidacloprid (IMI) and propoxur (PRO), an N–methylcarbamate compound. Nwani *et al.* [26] reported that TBARS levels increased in *Channa punctatus* individuals as a result of atrazine exposure. Sinhorin *et al.* [27] reported increases in TBARS levels in *Pseudoplatystoma sp*. as a result of glyphosate exposure. Rossi *et al.* [28] followed increase in TBARS levels in *Markiana nigripinnis* and *Astyanax lacustris* species with the effect of herbicide glyphosate (GLF), insecticide bifenthrin (BFT), cyproconazole (CYP) mixtures and fungicides azoxystrobin (AZ). Serdar *et al.* [21] observed increase in TBARS levels in *D. polymorpha* with the impact of Dimethoate (DMT) and Malathion (MLT) pesticides. Serdar [29] Dimethoate (DMT) pesticide observed increases in TBARS levels in *G. pulex*. Serdar *et al.* [30] determined increases in MDA levels in *D. polymorpha* due to dimethoate (DM) induced toxicity. Söylemez *et al.* [31] determined that beta–cyfluthrin (β–CF) produced increase in MDA levels in *D. polymorpha*. Bhattacharya *et al.* [32] determined that sodium laureth sulfate (SLES) caused increases in MDA levels in *Tubifex tubifex*. Increases in TBARS levels in *D. polymorpha* after klaxon exposure are thought to be related to the concentration and duration of exposure.

GSH level

GSH level decreased with increasing concentration compared to control (A). Groups B, C and D showed a statistically significant (P<0.05) decrease in both 24 and 96–h practice groups compared to the control (FIG. 2).

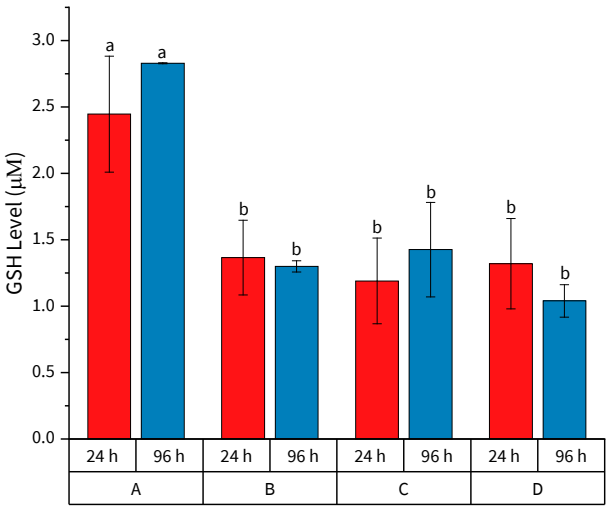

FIGURE 2. GSH (µM) levels of *Dreissena polymorpha* exposed to different konsantrasyon of klaxon. Different letters on the bars indicate a statistically significant difference between the groups within same treatment period (P<0.05)

Oxidative stress can be caused by a decrease in cellular GSH contents below a critical levels, which prevents xenobiotics from conjugating such as klaxon to GSH and thus allows them to freely associate covalently linked to cell proteins [33]. Ji *et al.* [24] reported decrease in GSH levels in *C. auratus* as a result of medium–term exposure to BKF alone and in combination with PCB118 and DDT. Ferreira *et al.* [34] reported that sublethal concentrations of tebuconazole (Teb), methyl parathion (MP) and a glyphosate–based herbicide (Gly) decreased GSH levels in *R. quelen* (Teleostei). Serdar *et al.* [21] observed decrease in GSH levels in *D. polymorpha* with the impact of DMT and MLT pesticides. Serdar [29] stated that DMT pesticide decreases caused in GSH levels in *G. pulex*. Serdar *et al.* [30] reported decrease in GSH levels in *D. polymorpha* due to DM–induced toxicity. Söylemez *et al.* [31] reported that beta–cyfluthrin (β–CF) caused decrease in GSH levels in *D. polymorpha*. In this study, decreases in GSH levels were detected with claxone exposure, which is in parallel with the studies in the literature.

SOD activity

SOD activity decreased as concentration increased compared to control (A). While groups C and D showed a statistically significant (P<0.05) decrease in both 24 and 96–h practice groups compared to control group, the decrease in group B was found statistically insignificant (P>0.05) (FIG. 3).

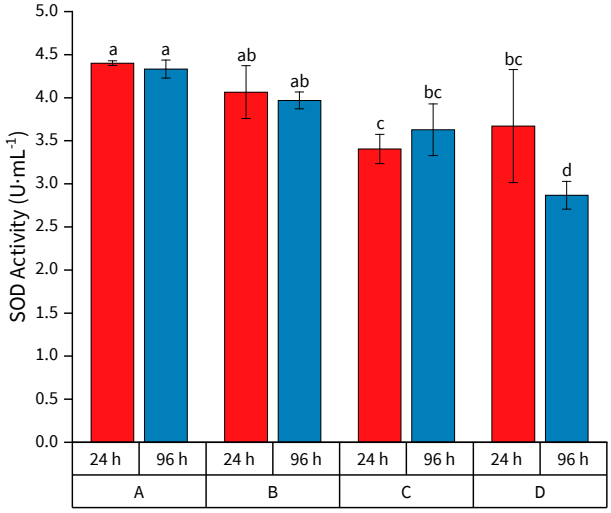

FIGURE 3. SOD (U·ml^{-1}) activities of *Dreissena polymorpha* exposed to different concentration of klaxon. Different letters on the bars indicate a statistically significant difference between the groups within same treatment period (P<0.05)

Superoxide dismutase (SOD) is an important enzyme in the antioxidant defense line and catalyzes the conversion of superoxide anion to hydrogen peroxide [22]. It is thought that the decreases in SOD activity in *D. polymorpha* at the end of klaxon exposure is due to catalyzing the conversion of superoxide anion to hydrogen peroxide. The results were tried to be supported by literature studies showing similar results. Rossi *et al.* [28] observed decreases

in SOD activities in *M. nigripinnis* and *A. lacustris* species with the effect of GLF, BFT, AZ and CYP mixtures. Serdar *et al.* [21] observed decrease in SOD activities in *D. polimorpha* with the effect of DMT and MLT pesticides. Serdar [29] stated that DMT pesticide caused decreases in SOD activities in *G. pulex*. Serdar *et al.* [30] reported decreases in SOD activities in *D. polymorpha* due to DM–induced toxicity. Cikcikoglu Yildirim *et al.* [35] observed that SOD activity increased with the effect of ibuprofen (IBU) and propranolol (PRO) using *Gammarus pulex*.

CAT activity

CAT activity decreased as concentration increased compared to control (A). While the 24–hour practice groups in group D showed a statistically significant (P<0.05) decreases compared to the control, the decreases in groups B and C was found statistically insignificant (P>0.05) (FIG. 4). In the 96–hour practice groups, statistically significant decreases (P<0.05) were detected in all groups compared to the control (A).

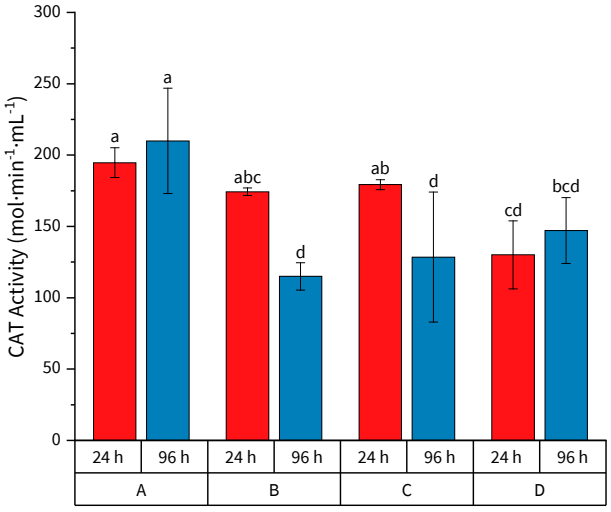

FIGURE 4. CAT (nmol·min^{-1}·ml^{-1}) activities of *Dreissena polymorpha* exposed to different concentration of klaxon. Different letters on the bars indicate a statistically significant difference between the groups within same treatment period (P<0.05).

Catalase catalyses the conversion of H_2O_2, produced during various metabolic processes, into less toxic molecules of water and oxygen [36, 37]. Concentration – and time–dependent decreases in CAT activity were detected in *D. polymorpha* individuals exposed to klaxon. In studies similar to these results, Marins *et al.* [38] examined the effect of pesticides in different tissues of *Oreochromis niloticus* and observed decreases in CAT activities. Marins *et al.* [25] observed increases in TBARS levels in *Rhamdia quelen* species exposed to imidacloprid (IMI) and propoxur (PRO), an N–methylcarbamate compound. Marins *et al.* [25] observed decreases in CAT activities in *Rhamdia quelen* species under IMI and PRO exposure. Ferreira *et al.* [34] reported decreases in GSH levels at sublethal concentrations of MP, Gly and Teb pesticides. Nwani *et al.* [26] reported increases in CAT activities in *C. punctatus* individuals as a result of atrazine exposure. Rossi *et al.* [28] observed decreases in CAT activities in *M. nigripinnis* and *A. lacustris* species with the effect of GLF, BFT, AZ and CYP mixtures. Serdar *et al.* [21] observed decreases in CAT activities in *D. polimorpha* with the effect of DMT and MLT pesricides. Serdar [29] stated that DMT pesticide caused decreases in CAT activities in *G. pulex*. Serdar *et al.* [30] reported decreases in CAT activities in *D. polymorpha* due to DM–induced toxicity. Aydın *et al.* [39] reported decreases in CAT activities in *D. polymorpha* due to the effect of Gamma Cyhalothrin (GCH) pesticide. CAT activity in *D. polymorpha* individuals exposed to klaxon was significantly lower in all experimental groups compared to control group organisms. This decrease is also supported by the literature.

GPx activity

GPx activity decreased as concentration increased compared to control (A). Groups B, C and D showed a statistically significant (P<0.05) decrease in both 24 and 96 hour practice groups compared to the control (FIG. 5).

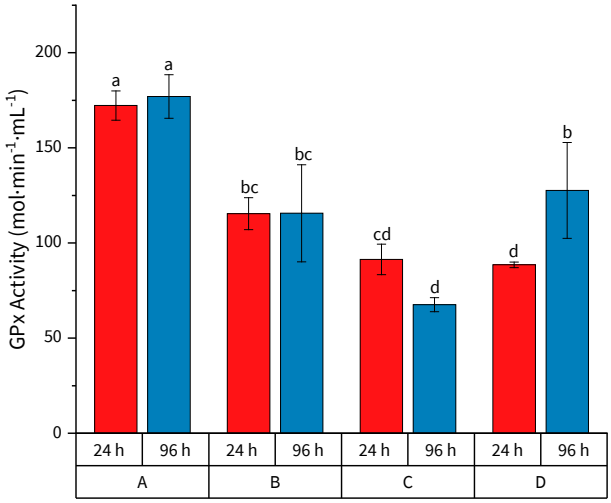

FIGURE 5. GPx (nmol·min^{-1}·ml^{-1}) activities of *Dreissena polymorpha* exposed to different doses of klaxon. Different letters on the bars indicate a statistically significant difference between the groups within same treatment period (P<0.05)

GPx is a catalyst for the reduce of lipid peroxides and hydrogen peroxide [40]. It has been shown that negative feedback caused by excess substrate or damage from oxidative modification reduces the activity of this enzyme. The inhibition of GPx activity could reflect a failing antioxidant system in the presence of plant protection products [41] or a direct effect of superoxide or plant protection products on enzyme synthesis [42]. Serdar [29] reported that DMT pesticide caused decreases in GPx activity in *G. pulex*. Serdar *et al.* [30] reported decreases in GPx activity in *D. polymorpha* due to DM–induced toxicity. Aydın *et al.* [39] reported changes in GPx activity in *D. polymorpha* due to the effect of Gamma Cyhalothrin (GCH) pesticide. GPx activity in *D. polymorpha* individuals exposed to klaxon was significantly lower

in all experimental groups compared to control group organisms. This decrease may indicate that hydroperoxide product synthesised by lipid peroxidation exceeds the antioxidant capacity.

CONCLUSION

In this study, some antioxidant enzymes and various markers of oxidative stress were evaluated and examined in *D. polymorpha* exposed to the xenobiotic klaxon. Klaxon exposure caused changes in the antioxidant defense system. In *D. polymorpha*, exposure to sublethal concentrations of klaxon, an increases in TBARS level, and decreases in GSH level, SOD, CAT, GPx activities were observed. These biomarkers were determined to be effective biomarkers in *D. polymorpha* with klaxon exposure.

Conflict of Interests

The author declare no competing interests

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