https://doi.org/10.52973/rcfcv-e35488 Revista Científica, FCV-LUZ / Vol. XXXV

The effect of Dimethoate on oxidative stress and antioxidant responses of *Pontastacus leptodactylus*

El efecto del dimetoato sobre el estrés oxidativo y las respuestas antioxidantes de *Pontastacus leptodactylus*

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ABSTRACT

Dimethoate (DMT) pesticide is one of the chemicals used to protect some agricultural areas from harmful organisms. DMT residues released directly or indirectly to the environment cause serious problems in nature. DMT residues mixed with the aquatic environment adversely affect aquatic organisms and this effect is carried to humans through the food chain. In this study, oxidative stress responses induced by DMT pesticide in *Pontastacus leptodactylus* were investigated. For this purpose, oxidative stress and antioxidant parameters Thiobarbituric acid reactive substances (TBARS), Glutathione (GSH), Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) caused by dimethoate (DMT) pesticide in *P. leptodactylus* at 17.5, 35, and 70 mg·L^{-1} concentrations at 24 and 96 hours were investigated. Results were determined using ELISA kits. No significant difference was observed in GSH levels and SOD activities compared to control. Statistically significant differences were observed between decreases in CAT and GPx activities and increases in TBARS levels. SPSS 24.0 package program one–way ANOVA (Duncan 0.05) was used in the evaluation of biochemical analyzes. As a result, it was determined that DMT caused oxidative stress formation in *P. leptodactylus* and caused changes in enzyme activities.

Key words: Dimethoate, *Pontastacus leptodactylus*, oxidative stress, antioxidant, biomarkers

RESUMEN

El pesticida dimetoato (DMT) es uno de los productos químicos utilizados para proteger algunas áreas agrícolas de organismos nocivos. Los residuos de DMT liberados directa o indirectamente al medio ambiente causan graves problemas en la naturaleza. Los residuos de DMT mezclados con el medio acuático afectan negativamente a los organismos acuáticos y este efecto se transmite a los humanos a través de la cadena alimentaria. En este estudio, se investigaron las respuestas al estrés oxidativo inducidas por el pesticida DMT en *Pontastacus leptodactylus*. Para ello, se investigaron el estrés oxidativo y los parámetros antioxidantes Sustancias reactivas al ácido tiobarbitúrico (TBARS), glutatión (GSH), superóxido dismutasa (SOD), catalasa (CAT) y glutatión peroxidasa (GPX) causados por el pesticida dimetoato (DMT) en *P. leptodactylus* en concentraciones de 17,5; 35 y 70 mg·L^{-1} a las 24 y 96 horas. Los resultados se determinaron utilizando kits de ELISA. No se observaron diferencias significativas en los niveles de GSH y las actividades de SOD en comparación con el control. Se observaron diferencias estadísticamente significativas entre disminuciones en las actividades de CAT y GPx y aumentos en los niveles de TBARS. Se utilizó ANOVA unidireccional del programa SPSS 24.0 (Duncan 0,05) en la evaluación de los análisis bioquímicos. Como resultado, se determinó que el DMT provocó la formación de estrés oxidativo en *P. leptodactylus* y provocó cambios en las actividades enzimáticas.

Palabras clave: Dimetoato, *Pontastacus leptodactylus*, estrés oxidativo, antioxidante, biomarcadores.

Received: 12/07/2024 **Accepted: 29/10/2024** **Published: 18/01/2025**

INTRODUCTION

Environmental pollution is the term used to describe all types of unnatural damage done to the environment by humans. Environmental pollution has an impact on all living organisms, including humans, at different times during their existence, from conception to death. Even though it is up to each individual to lessen these effects, environmental pollution is steadily rising as a result of financial worries [1]. Numerous new types of pollutants have entered the aquatic environment at the same time that global economic activity is expanding quickly [2, 3]. Natural water resources are susceptible to chemical intrusion from runoff, agricultural fields, and home and industrial wastewater [4, 5]. In agriculture as well as non–agricultural contexts including businesses, athletic fields, and other urban green spaces, pesticides including insecticides, herbicides, and fungicides are frequently used [6, 7]. Aquatic flora and animals, as well as the environment, are negatively impacted by pesticides in water [8]. Despite having a low concentration in the water matrix, persistent pesticides are more dangerous because of their high stability and bioaccumulative properties. The primary cause of high bioaccumulation in aquatic creatures is the high water solubility of particular pesticide groups [9].

Examining the harmful and destructive effects of physical or chemical agents on living things is known as toxicology Ödün and Serdar [10]. Finding the concentration at which a drug damages aquatic species is the goal of aquatic toxicology experiments in this context [11]. Oxidative stress, also known as oxygen free radicals and other reactive oxygen species (ROS), has grown to be a significant area of research for environmental toxicity investigations in biological systems. Different pollutants cause different mechanisms of toxicity, including oxidative damage to proteins, Deoxyribose Nucleic Acid (DNA), and membrane lipids as well as adjustments to antioxidant enzymes. As the initial line of defense, endogenous antioxidants including glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) may efficiently eliminate generated free radicals Bhattacharjee and Sil [12] and shield cells from oxidative injury. The most efficient biomarker in defense against oxidative stress in both vertebrates and invertebrates Batista *et al.* [13] is CAT activity, which is located in biological tissues and breaks down hydrogen peroxide into oxygen and water to protect tissues from oxidative damage [14]. The redox status of the cell is altered by reduction [15, 16].

Due to their susceptibility to pollution contamination in aquatic environments, it has been reported that crustaceans are species utilized for evaluation [17, 18]. Crustaceans are suited for use as water pollution biomarkers [19]. Its long life cycle, wide distribution and sedentary lifestyle make it a good bioindicator for heavy metals and organic pollutants. In crustaceans, the gill is a multifunctional and complex organ that is in close contact with the ambient water. Contaminants can accumulate significantly in the gills, and due to its lipophilicity, DMT can easily penetrate through the gill, causing oxidative stress and tissue damage [20, 21]. In this Country, it is widely used in the fight against insecticide and acaris pests in products such as olives (*Olea europaea*), pistachios (*Pistacia vera*), plums (*P. domestica*), apples (*Malus domestica*) and peaches (*Prunus persica*) by farmers already licensed is used. Dimethoate is one of the organophosphate insecticides widely used against various pests in many crops, and many studies have been conducted on the toxicity of dimethoate to aquatic and terrestrial organisms [22].

In this study, the effects of pesticide DMT, which is a frequent environmental pollutant, on Thiobarbituric acid reactive substances (TBARS), Glutathione (GSH) levels, and Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) enzyme activities in the hepatopancreatic tissue of freshwater lobster *P. leptodactylus* were investigated.

MATERIALS AND METHODS

Test organism

P. leptodactylus, a freshwater lobster used in the test, was acquired from a business engaged in aquaculture.

Adaptation of test organisms to the laboratory environment

P. leptodactylus and water from the location where it was obtained were transported to the lab in plastic boxes. It was brought as quickly as possible to the lab to lessen the strain on the living things. They spent about a month getting used to the lab environment. 15 ± 2°C for the ambient water temperature; a constant photoperiod of 14:10 light to dark hours. Stock tank abiotic variables (dissolved oxygen: 11.52 ± 0.87 mg·L^{-1}; pH: 8.14 ± 0.4; electrical conductivity: 478 ± 76 S·µcm^{-1}; salinity: 0.3 ± 0.02 µg·L^{-1}) were tested daily with a YSI professional plus brand multiparameter device and changes were recorded. Every day, the water quality in each aquarium was changed to prevent further stress from deteriorating water. Feeding took place once every day. Each tank had its leftover food and waste removed, and freshwater was added every day to replenish the aquatic ecosystem. PVC pipes were added to stock tanks as shelter for the well–being of the crayfish.

Chemical substance supply

DMT pesticide containing 400 g·L^{-1} dimethoate active ingredient was purchased from a commercial (Koruma) company that markets agricultural products.

Determination of sublethal concentrations

DMT is applied at a rate of 80 to 140 g·L^{-1} in agricultural regions [23]. Sublethal concentrations were established, as in all toxicological investigations, by taking into account the application concentrations discovered in our DMT application study, the release rates to the environment, and the application concentrations compared to their values in this range.

Research design

Each experimental group was placed in a glass tank with a capacity of 80 L with 30 L of water with the specified properties and 6 model animals were placed. The control group was created with no pesticide, the second group with a pesticide concentration of 17.5 mg·L^{-1}, the third group with a pesticide concentration of 35 mg·L^{-1} and finally with a pesticide concentration of 70 mg·L^{-1} (FIG. 1). These conditions (exposure time) were maintained for 96 hours (h). A summary of the experimental design is presented below.

» C1 Group (Control); administration group: no DMT was present.

» C2 Group; was exposed to DMT concentrations of 17.5 mg·L^{-1} for 24 and 96 h.

» C3 Group; was exposed to DMT concentrations of 35 mg·L^{-1} for 24 and 96 h.

» C4 Group; was exposed to DMT concentrations of 70 mg·L^{-1} for 24 and 96 h.

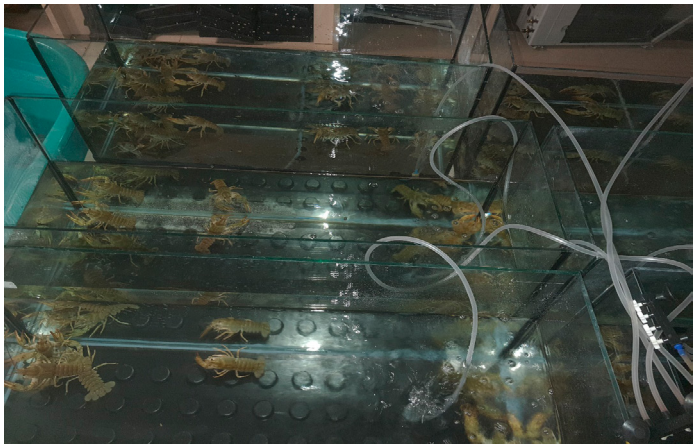

FIGURE 1. Experimental applications of *Pontastacus leptodactylus* exposed to DMT. (All experiments in the experimental study were performed three times)

Biochemical evaluation

In the experimental groups (including 3 replicate groups conducted simultaneously), three test organisms were randomly chosen from the aquarium. The animals from which the hepatopancreas samples would be taken were placed in ice water for 30 min, underwent cold shock treatment, and had a 0.5 g sample of the organ removed from each living item. To assess antioxidant properties, the samples were weighed (BEL engineering, M214Ai model precision scale, Italy), homogenized (Daihan brand, Hg–15D Digital ultraturax model, Korea), and 1/5 w/v of PBS buffer (phosphate–buffered saline solution) was added. The samples were centrifuged (NUVE brand, NF1200R model centrifugal, Turkiye) for 15 min at 54,000 G. Until measurements were taken, supernatants were stored at -86°C in a deep freezer (Daihan brand, Wisd ultra freezer model, Korea). SOD, CAT, and GPx activities as well as GSH and Thiobarbituric acid reactive substances (TBARS) levels were measured using an ELISA (Agilent brand, BioTek 800 TS Absorbance Reader, USA) reader. In this investigation, the biochemical reaction was determined by measuring the GSH, TBARS level, SOD, CAT, and GPx activities. The SOD, CAT, and acetylcholinesterase (AChE) kits utilized in the study were bought from a business called CAYMAN. TBARS: 10009055, GSH: 703002, CAT: 707002, SOD: 706002, and GPx: 703102 are the respective catalog numbers for this kit.

Statistical analysis

In this study, SPSS version PASW Statistics 24.0 was used for statistical analysis. One–way ANOVA and Duncan's multiple range tests were applied to determine statistical differences in the control and all exposure groups (A, B, C, D) (a,b,c,d,e $P<0.05$). Two–tailed independent t–test was applied to compare differences between exposure times (24 and 96 h) in the same control and exposure groups ($P<0.05$).

RESULTS AND DISCUSSION

In the literature, there are many scientific studies investigating the effects of pollutants on aquatic organisms with various biomarkers. Oxidative stress represents the potential for mismatch between ROS production and removal and damage to tissues and cellular components and is generally accepted in toxicology studies [8].

Thiobarbituric acid reactive substances level

Different concentration and time dependent TBARS levels of DMT are given in FIG. 2. It was determined that there was a statistically significant increase ($P<0.05$) in the TBARS level in the C3 and C4 groups in the treatment groups exposed to DMT compared to the control, and there were statistically significant increases in the C1 and C4 groups at the 96th hour in terms of duration (24 and 96 h) has been done.

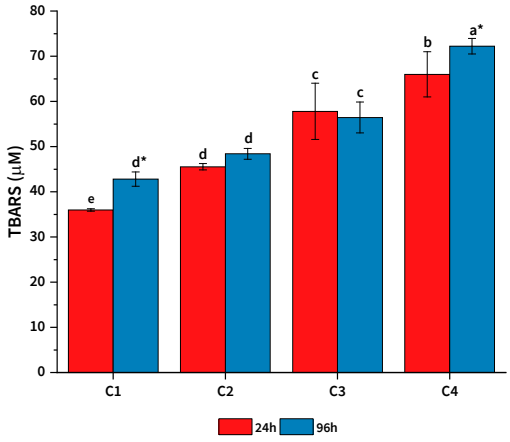

FIGURE 2. TBARS (µM) levels of *Pontastacus leptodactylus* exposed to dimethoate, Different letters on the column are statistically significant ($P<0.05$). * indicates time (24 and 96 h) difference

The decrease in Glutamate–Cysteine Ligase Catalytic Subunit (GCLC) protein synthesis, which provides the production of SOD, GPX, GST and GSH, leads to a decrease in the antioxidant defense of the cell and thus an increase in the amount of TBARS. Lidova *et al.* [24], in their study, *Procambarus fallax* f. *virginalis* also examined the oxidative stress parameters of cypermethrin and stated that TBARS level decreased as a result. Huang *et al.* [25], examined the oxidative stress parameters of *Procambarus clarkii* of Imidacloprid and observed a significant increase in increases in MDA levels. Yüksel *et al.* [8], examined the bioresponse of malathion pesticide on *Gammarus pulex* and as a result, increases in TBARS levels. Rossi *et al.* [26], investigated the oxidative stress responses of the herbicide glyphosate, insecticide bifenthrin, BF and fungicide azoxystrobin (AZ) and cyproconazole (CYP), mixtures in Markiana living in rice fields, and as a result, they increses in TBARS levels. It is thought that the increase in TBARS level in *P. leptodactylus* with

the effect of DMT is due to the decrease in antioxidant defense in the cell. It was determined that the TBARS level increased with the increase in zaman and DMT concentration. The increased TBARS levels reflect the increase lipid peroxidation (LPO) found in the present investigation, which may have resulted from an increase of free radicals as a result of stress condition generated by pesticide exposure.

Glutathione level

Different concentration and time dependent GSH levels of DMT are given in FIG. 3. It was determined that there was no significant increase in the GSH level in the treatment groups exposed to DMT compared to the control (P<0.05), and there was no significant increase in the GSH level in the comparison in terms of time (24 and 96 h) (P<0.05).

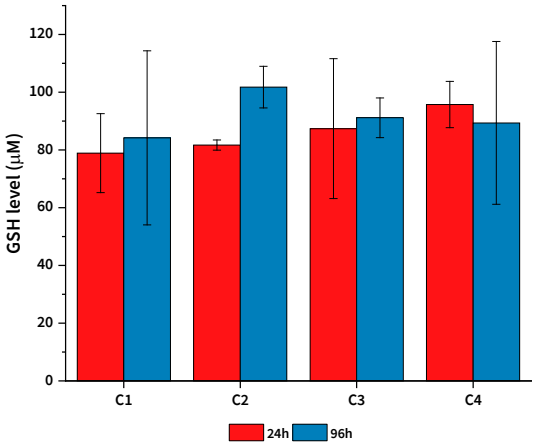

FIGURE 3. GSH (µM) levels of *Pontastacus leptodactylus* exposed to dimethoate

Lidova *et al.* [24], examined the oxidative stress parameters in the model organism *P. fallax* f. *virginalis* exposed to cypermethrin and stated that the GSH level decreased as a result. Serdar [27], evaluated the oxidative stress parameters in *G. pulex*, which was exposed to DMT, and stated that there was no change in GSH levels. Söylemez *et al.* [28], investigated some biochemical responses of Beta–Cyfluthrin (β–CF) in *Dreissena polymorpha* and stated that GSH level decreased as a result. Zhang *et al.* [29], studied the protective effects of Melatonin and oxidative damage of Chinese mitten crab (*Eriocheir sinensis*) exposed to deltamethrin and noted that GSH content increased in the organisms exposed to deltamethrin. Lin *et al.* [30], investigated the effect of ammonia on *P. clarkii* and as a result, decreases in GSH levels observed. Abd El–Atti *et al.* [31], examined the oxidative stress parameters of titanium dioxide in *P. clarkii* and observed increases in GSH levels as a result. Similar to the current study, it was reported that there was a decrease in several aquatic organisms in GSH content produced by anticholinesterase agents [32]. GSH depletion is associated with the oxidation of glutathione peroxidases due to an increase in free radicals and/or direct oxidation of these compounds [33]. Additionally, decreasing GSH content can be associated with its role as GST substrate in detoxification reactions.

Superoxide dismutase activity

Different concentration and time dependent SOD activity of DMT is given in FIG. 4. It was determined that there was no significant increase in SOD activity in the treatment groups exposed to DMT compared to the control (P<0.05), and there was no significant increase in SOD activity in terms of duration (24 and 96 h) compared to the control (P<0.05).

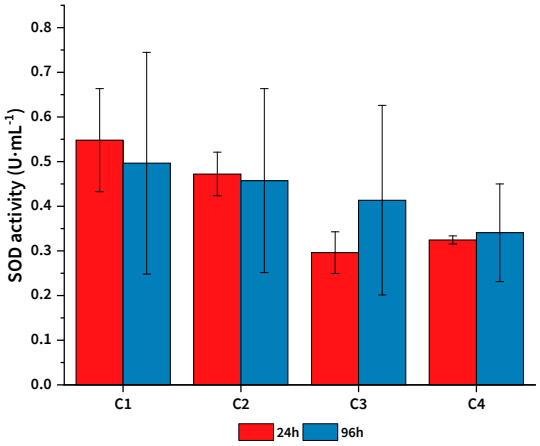

FIGURE 4. SOD (U·ml^{-1}) activities of *Pontastacus leptodactylus* exposed to dimethoate, different letters on the column are statistically significant (P<0.05)

While the SOD enzyme converts the superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2). SOD, a group of metalloenzymes, is the primary defense against the toxic effects of superoxide radicals in aerobic organisms and catalyses the transformation of superoxide radicals into H_2O_2 and O_2 which play an important role in antioxidant system cleaning**.** The response of the antioxidant system to oxidative stress in various tissues differs from species to species due to differences in antioxidant potentials of these tissues. The changes in SOD activities in *P. leptodactylus* individuals exposed to DMT are thought to depend on the concentration of the pollutant. The decreases in SOD enzyme activity determined as a function of time and concentration are indicative of a defense mechanism developed by the model organism against oxidative stress caused by the pollutant DMT. The toxic effect decreased with increasing concentration and time. Ghisi *et al.* [34], examined the effect of *P. clarkii* exposed to prometrine on oxidative stress and antioxidant response and reported that prometrine caused a decrease in SOD activity. Abdel–Daim *et al.* [31], examined the oxidative damage caused by chlorpyrifos in *Oreochromis niloticus* exposed to chlorpyrifos and reported that as a result, SOD values decreased. Nataraj *et al.* [35], investigated hepatic oxidative stress in freshwater fish *Labeo rohita* exposed to Profenofos, and as a result, they found significant reductions in SOD activity. Uçkun and Öz [36], investigated the oxidative stress caused by Azoxystrobin in *Astacus leptodactylus* and stated that as a result, the level of SOD in the gills and muscles decreased. Yang *et al.* [37], examined the oxidative stress effects of *P. clarkii,* which they exposed to the herbicide atrazine, and stated that SOD were inhibited. Lidova *et al.* [24], in their study, *P. fallax* f. *virginalis* also examined the oxidative stress parameters of cypermethrin and stated that

SOD activity decreased as a result. Yang *et al.* [37], examined Cyhalofop–butyl and pyribenzoxim–induced oxidative stress in *P. clarkii* muscle and found decreases in SOD activity. Huang *et al.* [25], examined the oxidative stress parameters of *P. clarkii* of Imidacloprid and observed a significant increase in SOD activity. Serdar [27], evaluated the oxidative stress parameters in *G. pulex*, which was exposed to DMT, and stated that there was no decrease in SOD activity, Zhang *et al.* [29], examined the oxidative damage of the Chinese mitten crab (*E. sinensis*) exposed to Melatonin deltamethrin and stated that the SOD activity decreased. Lin *et al.* [30], investigated the effect of ammonia on *P. clarkii* and as a result, decreases in SOD activity were observed.

Catalase activity

The CAT activity of DMT depending on different concentrations and time is given in FIG.5. The changes in CAT activity of 24 and 96 hour exposure groups (C2, C3 and C4) were determined to be statistically significant compared to the control (C1) (P<0.05). When the changes of the same application groups at different times (24 and 96 h) were compared, it was determined to be statistically insignificant (P>0.05).

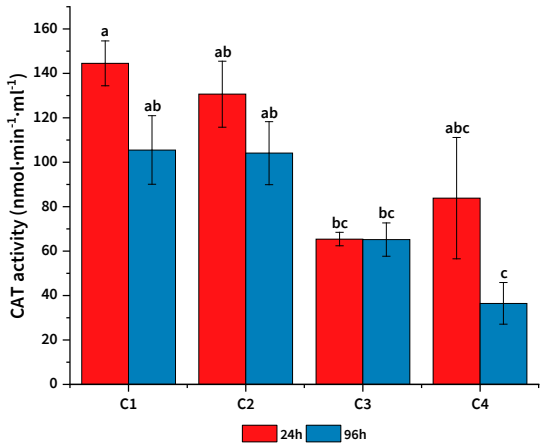

FIGURE 5. CAT (nmol·min^{-1}·ml^{-1}) activities of *Pontastacus leptodactylus* exposed to dimethoate, different letters on the column are statistically significant (P<0.05)

Catalase is the main enzyme for the detoxification of ROS and it is a very common enzyme found in almost all living organisms using oxygen. It catalyses the decomposition of hydrogen peroxide affects the generation of water and oxygen [32, 33]. It can be said that the decrease in CAT enzyme activity in parallel with the increasing concentration groups compared to the control group is an indicator of a defense mechanism developed by the model organism against oxidative stress induced by the pollutant DMT and decreases in parallel with the increasing concentration. Ghisi *et al.* [34], examined the effect of *P. clarkii*, which they applied *Prometrine herbicide*, on oxidative stress and antioxidant response, and stated that there was a decrease in CAT activity as a result of the study. Abdel–Daim *et al.* [31] study, examined the oxidative damage caused by chlorpyrifos toxicity in *Oreochromis niloticus*, and as a result, they determined decreases in CAT values. Nataraj *et al.* [35], investigated hepatic oxidative stress in freshwater fish *Labeo rohita* exposed to Profenofos, and as a result, they found significant reductions in CAT activity.

Uçkun and Öz [36], investigated the oxidative stress caused by azoxystrobin in *A. leptodactylus* and stated that as a result, the level of GSH in the gills and muscles decreased. Yang *et al.* [37], examined the oxidative stress effects of *P. clarkii,* which they exposed to the herbicide atrazine, and stated that CAT were inhibited. Yang *et al.* [37], examined cyhalofop–butyl and pyribenzoxim–induced oxidative stress in *P. clarkii* muscle and found decreases in CAT activity. Huang *et al.* [25], examined the oxidative stress parameters of *P. clarkii* of Imidacloprid and observed a significant increase in CAT activity. Yüksel *et al.* [8], examined the bioresponse of malathion pesticide on *G. pulex* and as a result, determined reductions in CAT activity. Serdar [27], evaluated the oxidative stress parameters in *G. pulex*, which was exposed to DMT, and stated that there was no decrease in CAT activity. Rossi *et al.* [26], investigated the oxidative stress responses of the herbicide glyphosate, insecticide bifenthrin, BF and fungicide azoxystrobin, AZ and cyproconazole, CYP mixtures in Markiana living in rice fields, and as a result, they observed decreases in CAT activity and It is thought that the decreases in CAT activity in *P. leptodactylus* with the effect of DMT is due to the decrease in antioxidant defense in the cell. Gao *et al.* [38], investigated the oxidative stress results of Maduramycin in *P. clarkii* and stated that there were decreases in CAT activity. Lin *et al.* [30], investigated the effect of ammonia on *P. clarkii* and as a result, decreases in CAT activity were observed.

Glutathione peroxidase activity

GPx activities in *P. leptodactylus* exposed to DMT concentrations over time are given in FIG. 6. A statistically significant (P<0.05) decrease was detected in the GPx activity of the 24 and 96 h exposure groups compared to the control group (FIG. 6).

Huang *et al.* [25], examined the oxidative stress parameters of *P. clarkii* of Imidacloprid and observed a significant increase in GPx activity increases. Yüksel *et al.* [8], examined the bioresponse of malathion pesticide on *G. pulex* and as a result, determined reductions in GPx activity. Serdar [27], evaluated the oxidative

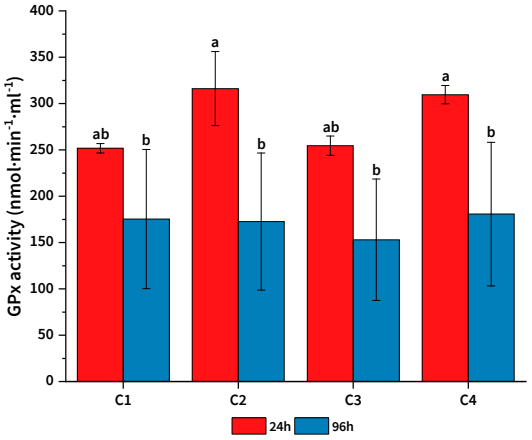

FIGURE 6. GPx (nmol·min^{-1}·ml^{-1}) activities of *Pontastacus leptodactylus* exposed to dimethoate, different letters on the column are statistically significant (P<0.05)

stress parameters in *G. pulex*, which was exposed to DMT, and stated that there was nno change in GPx aktivity. Gao *et al*. [38], investigated the oxidative stress results of Maduramycin in *P. clarkii* and stated that there were decreases in GPx activity.

The oxidative stress and antioxidant responses in this study were related to the DMT concentrations determined in the hepatopancreas, which is thought to be dependent on the concentration and exposure time. Since the data obtained in this study are consistent with the study data in the literature, it is thought that it will contribute to the literature.

CONCLUSION

It was determined that DMT has a toxic effect on P. leptodactylus. When the study's findings were assessed, biomarker parameters (SOD, CAT and GPx activty and TBARS, GSH level) responses of DMT pesticide in P. leptodactylus were identified. It has also been shown that biochemical parameters such as SOD, CAT, GPx activities and GSH, TBARS levels used are suitable biomarkers for the evaluation of the toxic effects of DMT.

Credit authorship contribution statement

Ayse Nur Aydın: Visualization, Validation, Supervision, Formal analysis, Writing – review & editing. Hilal Bulut: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Osman Serdar: Validation, Formal analysis, Data curation, Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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