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Effect of naproxen on oxidative stress biomarkers in *Gammarus pulex*

Efecto del naproxeno sobre los biomarcadores de estrés oxidativo en *Gammarus pulex*

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are a widely prescribed medication class that is used in the treatment of numerous conditions worldwide. Of these drugs, naproxen is the most commonly used NSAID. Following administration, non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen are eliminated from the body in either their original chemical form or as metabolites, ultimately entering the aquatic environment. The current study sought to show the impacts of naproxen on the oxidant/antioxidant status of *Gammarus pulex,* an aquatic invertebrate (Amphipoda). *Gammarus pulex* were exposed to sublethal concentrations of naproxen (3.44, 6.87 and 13.75 mg·L^{-1}) for 96 hours (h). Whole body tissue samples were collected after 24, 48 and 96 h of exposure and analysed to determine the oxidant/antioxidant status by quantifying malondialdehyde (MDA) and total glutathione levels (GSH), and superoxide dismutase (SOD), and catalase (CAT) activities of the *G. pulex*. The level of MDA exhibited a remarkable increase, while the endogenous GSH level showed a significant depletion in tested whole body tissues in a time-dependent manner after naproxen treatment of *G. pulex*. In *G. pulex* exposed to the highest dose of naproxen; decreases in GSH activity, SOD and CAT activities were observed.The SOD activity did not show a discernible rise in statistics after 24 and 48 h of exposure, however, a difference was observed after 96 h compared to the control group (P<0.05). The findings of this study demonstrated the capacity of naproxen to initiate oxidative stress and elevate MDA levels in *G. pulex*, even at remarkably low concentrations. This study emphasizes that it is essential to develop effective methodologies to impede naproxen entry into the aquatic environment.

Key words: *Gammarus pulex*, naproxen, oxidative stress, antioxidant status

RESUMEN

Los antiinflamatorios no esteroideos (AINEs) son medicamentos ampliamente prescritos en todo el mundo y pertenecen a una clase terapéutica destacada. De estos fármacos, el naproxeno es un AINE de uso común. Después de la administración, los AINEs como el naproxeno se eliminan del cuerpo ya sea en su forma química original o como metabolitos, y finalmente pasan al medio acuático. El presente estudio buscó demostrar los impactos del naproxeno en el estado oxidante/antioxidante de *Gammarus pulex*, un invertebrado acuático (anfípodos). Los *G. pulex* estuvieron expuestos a concentraciones subletales de naproxeno (3,44, 6,87 y 13,75 mg·L^{-1}) durante 96 horas (h). Se recogieron muestras de tejido corporal entero después de 24, 48 y 96 h de exposición y se analizaron para determinar el estado oxidante/antioxidante cuantificando los niveles de malondialdehído (MDA) y glutatión total (GSH), y las actividades de superóxido dismutasa (SOD) y catalasa (CAT) de *G. pulex*. El nivel de MDA mostró un aumento notable, mientras que el nivel de GSH endógeno mostró una disminución significativa en los tejidos corporales completos analizados de manera dependiente del tiempo después del tratamiento con naproxeno de *G. pulex*. En *G. pulex* expuesta a la dosis más alta de naproxeno; Se observaron disminuciones en la actividad de GSH, SOD y CAT. La actividad de SOD no mostró un aumento perceptible en las estadísticas después de 24 y 48 h de exposición, sin embargo, se observó una diferencia después de 96 h en comparación con el grupo de control (P<0,05). Los hallazgos de este estudio demostraron la capacidad del naproxeno para iniciar el estrés oxidativo y elevar los niveles de MDA en *G. pulex*, incluso en concentraciones notablemente bajas. Este estudio enfatiza que es esencial desarrollar metodologías efectivas para impedir la entrada de naproxeno en el ambiente acuático.

Palabras clave: *Gammarus pulex*, naproxeno, estrés oxidativo, estado antioxidante

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INTRODUCTION

The Gammarus family, Gammaridae: Amphipoda, drew the attention of experts in taxonomy and ecology but in recent years, professionals in molecular biology, genetics, agriculture, and notably, toxicology also have been interested. *Gammarus* species have rapidly gained use in ecotoxicology due to their high abundance [1], distinct sexual dimorphism, ease of collection, and tolerance to a wide range of toxic substances [2, 3]. While the primary focus of research within the domain of aquatic toxicology currently centers on identifying sensitive long-term markers for a variety of test species including gammarids, the role in oxidative stress of acute toxicity exposure continues its popularity in elucidating the effects of toxins [1, 4, 5]. The assessment of chemical screening and the compliance of wastewater with discharge regulations is primarily based on physiological toxicity data. This approach plays a critical role in determining the potential effects of wastewater on human health and the environment.

The common use of non-steroidal anti-inflammatory drugs, including naproxen and other chemicals has been documented in various regions all around the world [6]. Naproxen, the propionic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID), widely used for the treatment of primary dysmenorrhoea, rheumatoid arthritis, osteoarthritis, ankylosing, tendinitis, bursitis, acute gout and juvenile arthritis in human [7, 8]. These applications have disrupted the balance within aquatic ecosystems, containing rivers, streams, lakes, estuaries, and coastal and deep oceans. Although it is known the pollution of NSAIDs, their potential toxic impacts on aquatic organisms have become the subject of research and experiment recently. Whereas the mechanisms of action of these substances are well-established in humans and other vertebrates [9], they remain largely unknown in aquatic invertebrates, that are continuously exposed to these compounds. As a result, there is considerable uncertainty regarding the environmental risks related to their presence [10]. The primary way NSAIDs work is by inhibiting the enzyme cyclooxygenase (COX). This enzyme is essential for converting arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. The therapeutic benefits of NSAIDs result from the reduced production of these eicosanoids [9]. Studies on the effects of naproxen on aquatic organisms have primarily concentrated on planktonic species such as water fleas (*Daphnia magna* and *Moina macrocopa*) and fish (*Oryzias latipes* and *Danio rerio*) [11, 12, 13]. Exposure to naproxen has been shown to affect the genetic material, inflammatory processes, and metabolic processes of aquatic organisms [13]. Additionally, it has been reported that naproxen poses an ecological risk to *Daphnia manga* [14].

Nonsteroidal anti-inflammatory drugs (NSAIDs) which encompass analgesic compounds constitute a highly notable category of pharmaceuticals globally with an approximate annual production of multiple kilotons [15]. Until now, the studies have indicated the presence of NSAIDs in the aquatic ecosystem, specifically in wastewater and surface water [16]. Naproxen is a kind of NSAID drugs. Ever since its introduction to the market in 1976, naproxen has consistently remained highly popular [17]. Induction of oxidative stress and the impact of detoxification mechanisms by various NSAIDs in aquatic organisms have also been evaluated through the measurement of activities of enzymes taking part in a variety of biochemical pathways [18, 19, 20, 21, 22, 23].

Oxidative stress happens as a consequence of an imbalance between the generation of reactive oxygen species (ROS) and the detoxification of these reactive compounds within an organism. ROS are naturally produced as byproducts of various essential biochemical reactions that play crucial roles in energy transfer, cellular defense, and cell signaling [24]. ROS have the potential to harm vital biological macromolecules and induce peroxidation of membrane lipids in biological systems, leading to the disruption of membrane structure and function. The exposure to environmental stressors can trigger a disproportionate increase in ROS levels, resulting in cellular and tissue damage [25]. Maintaining a balance between oxidants and antioxidants is a crucial for cellular homeostasis.

Gammarus pulex is a tiny amphipod crustacean that inhabits freshwater environments throughout Europe. This species is well-suited for biomonitoring studies due to its notable role in freshwater ecosystems. *G. pulex* serves as a vital food source for various invertebrates, fish, and birds, making it an important link in the food chain [26]. This organism has also been widely utilized in monitoring contaminants, including toxicity tests for a range of pollutants like metals, PAHs/PCBs, and pharmaceutical substances. This highlights its crucial role in assessing environmental risks [27, 28, 29, 30].

The current investigation aims to contribute to the assessment of aquatic toxicity tests employed for evaluating the potential toxicological influence of naproxen on aquatic organisms. Regarding that all effects are not necessarily detrimental, the primary objective of these tests is to identify chemicals that may have adverse impacts. The bioassay tests will yield a database that can be utilized to evaluate the risks associated with a given scenario.

MATERIALS AND METHODS

Living material

A model invertebrate species, *G. pulex* (L., 1758), was used for the exposure experiment. *G. pulex* samples were collected from the Munzur River (39.156820 N, 39.499640 E) in Tunceli province, from the slower flowing and relatively deeper parts of the source, using hand nets from under leaves and stones. *G. pulex* samples, which were quickly brought alive to the laboratory in air-reinforced plastic bottles, were placed in two 80×40×25 cm aerated stock aquariums prepared similar to their natural environment. Rested water taken from the natural environments where the samples were obtained was placed in the aquariums, and 50% of the water in the aquariums was replaced with rested water once a week.

Experimental setup and naproxen exposure

Before being used in experiments, *G. pulex* samples were fed with rotten willow tree leaves collected from their natural habitat in a room kept at a constant temperature of 18°C, in a 12:12 h light:dark cycle, for 15 days [28]. During adaptation, the feeding and mobility of the creatures were observed.

In the study, after 15 days of adaptation, *G. pulex* samples selected from stock aquariums; Care was taken to ensure that they were male individuals who had reached sexual maturity, completed their molting, were in good health, and had an average weight of approximately 0.350–0.400 g.

Gammarus pulex samples were divided into four groups after a two-week adaptation period to laboratory conditions. The experimental setup included a control and three experimental groups, each consisting of three replicates. 2 liter glass jars were

used in the experiments. 1 liter of water was placed in these glass jars and ventilated with air engines. 60 live *G. pulex* were used for all concentration groups and replicates, including the control group. Sublethal concentrations of naproxen were selected based on the 96-hour median lethal concentration (LC50) value previously reported for *G. pulex*, which was found to be 110 mg·L^{-1} by [31]. Naproxen was dissolved in 0.9% NaCl [32]. *G. pulex* was exposed to sublethal concentrations of naproxen at approximately 1/32, 1/16, and 1/8 of the 96-hour LC50 of naproxen (3.44; 6.87 and 13.75 mg·L^{-1}) for 24, 48, and 96 h. Experimental aquariums were ventilated during the exposure period and feeding was not done during the experiments. No deaths in animals were observed during these exposures.

- (Control Group), the control group drug-free.
- (A Group), 1/8 ratio of the LC50 value of naproxen.
- (B Group), 1/16 ratio of the LC50 value of naproxen.
- (C Group), 1/32 ratio of the LC50 value of naproxen.

Test organisms were exposed to naproxen for 24, 48 h and 96 h. At the end of these periods, 20 *G. pulex* from each experimental group were selected by random sampling for biochemical analyses.

Preparation of *G. pulex* homogenates and Biochemical estimations

At the end of the test, *G. pulex* were anaesthetized in icecold chilled water and whole bodies were isolated for biomarker analysis.

After rinsing with cold 0.09% NaCl solution and filtering to remove the liquid, 5 g of 20 *G. pulex* samples were weighed and used to prepare homogenate. Preparation of *G. pulex* homogenates involved homogenizing the tissues using a Teflon-glass homogenizer in a buffer solution containing 1.15% KCl, resulting in a 1:10 (w·v^{-1}) whole homogenate. Subsequently, the homogenates were centrifuged (Nüve, NF800R, Turkey) at 18000 G for 30 min at 4°C. The resulting supernatant was used for the analysis of malondialdehyde (MDA) and reduced glutathione (GSH) concentrations, as well as the determination of catalase (CAT), and superoxide dismutase (SOD) activities.

MDA level

MDA was measured in accordance with the method of Ohkawa *et al*. [33]. The MDA level was assessed using the thiobarbituric acid reactive substances (TBARS) method based on the reaction between MDA and thiobarbituric acid. To start the procedure, 0.1 mL of the extract was mixed with 0.15 mL of 0.8% thiobarbituric acid. Then, 0.04 mL of 8.1% sodium dodecyl sulphate and 0.15 mL of acetic acid were added sequentially. The resulting mixture was brought to a total volume of 0.5 mL with distilled water and then incubated (Nüve EN 400, Turkey) in a hot water bath at 95°C for 1 h. After cooling, 0.1 mL of distilled water and 0.5 mL of a mixture of n-butanol and pyridine (15:1 ratio, v·v^{-1}) were added. The mixture was vortexed (Nüve VM 02,Turkey) vigorously and after centrifugation at 4000 r·min^{-1} for 10 min, the absorbance of the organic layer (top layer) was measured at 532 nm using a spectrophotometer (Thermo Scientific, Multiskan GO, USA), distilled water was used as a blank. The amount of MDA was calculated using an extinction coefficient of 1.56 mM^{-1}·cm^{-1} and expressed in nmoles MDA·mg^{-1} protein.

GSH activity

GSH level will be determined using the method reported by Ellman [34]. Accordingly, Ellman's reagent and disodium hydrogen phosphate (Na_2HPO_4) will be added to the supernatants obtained from tissue samples centrifuged with precipitant solution (metaphosphoric acid, ethylenediaminetetetraacetic acid (EDTA), sodium chloride (NaCl) at 3000 rpm for 20 min. The mixture will be read in a spectrophotometer against the blank at 412 nm and and glutathione content was calculated from the standard curve.

CAT activity

The method described by Aebi [35] was employed to determine catalase activity. For this, 0.2 mL of tissue samples were taken, and 1 mL of hydrogen peroxide was added. The difference in absorbance at 0 and 30 s was measured at 240 nm. CAT activity was expressed as units·mg^{-1} protein·min^{-1}.

SOD activity

The Beauchamp and Fridovich [36] standard superoxide dismutase assay was performed using xanthine oxidase in combination with nitroblue tetrazolium (NBT). In addition, catalase was added at the rate of one unit per millilitre. The concentration of xanthine oxidase was meticulously adjusted to ensure roughly equivalent initial rates of blue formazan production under different experimental conditions. SOD activity was expressed in U·mL^{-1} of protein. U—one unit of SOD activity—corresponds to the amount of enzyme causing 50% inhibition of the rate of NBT reduction reaction.

Protein concentration, for calculating biomarker data were measured according to Lowry *et al*. [37].

Statistical analysis

All data are given as mean and standard deviation. Analysis of variance (ANOVA) was utilized to examine the obtained data and Tukey test, and differences between groups were determined. Statistical differences in all analyzes were determined at the 95% confidence interval. Statistical analyzes were performed using a commercial statistical program package (SPSS 25.0). To reduce the impact of random error on the results, the same protein preparation was used in all experiments. This ensured that any deviation from perfect linearity in the original preparation would be consistent across all trials. Thus, differences between data sets would more accurately reflect their inherent effects (if any) on each test.

RESULTS AND DISCUSSION

The results of MDA are shown in TABLE I. The TABLE I showed that there was a noticeable dose-dependent rise in the concentration of lipid peroxidation products (MDA) when *G. pulex* were exposed to naproxen. Notably, remarkable changes in the levels of lipid peroxidation products were monitored in contrast to the control group (P<0.05). Regarding the products of lipid peroxidation, the highest dosage (13.75 mg·L^{-1}, Group 4) showed a significant elevation after 24, 48 and 96 h of exposure compared to the control group measurement (P<0.05).

TABLE II provided a clear evidence of a conspicuous change in the activity of superoxide dismutase (SOD) in response to naproxen exposure in *G. pulex*. The whole body tissue of *G. pulex* exhibited a dose-dependent increase in SOD activity, and a remarkable change compared to the control group was observed at the applied dose of 13.75 mg·L^{-1} naproxen (P<0.05).It is vital that no deaths were recorded among *G. pulex* throughout the entire treatment period at this dose.

TABLE I
Malondialdehyde (μmol·g^{-1} protein, mean ± standard deviation) in 24–96 hours naproxen exposed and controls *Gammarus pulex*

	24 hours	48 hours	96 hours
Control Group	0.48 ± 0.02	0.63 ± 0.07	0.54 ± 0.04
A Group	0.68 ± 0.07^{a}	0.70 ± 0.04^{a}	0.84 ± 0.02^{a}
B Group	0.61 ± 0.00^{a}	0.81 ± 0.06^{a,b}	0.93 ± 0.05^{a,b,c}
C Group	0.74 ± 0.03^{a}	0.95 ± 0.18^{a,b}	0.79 ± 0.02^{a}

Different letters express significative differences (P<0.05). $^{a, b, c}$: A statistically significant difference exists between the mean (± SE) values represented by different letters on the same column. (P<0.05). ^{a}: Control Group, ^{b}: A Group, ^{c}: B Group

TABLE II
Superoxide dismutase activity (U mL^{-1}, mean ± standard deviation) in 24–96 hours naproxen exposed and controls *Gammarus pulex*

	24 hours	48 hours	96 hours
Control Group	6.28 ± 0.33	5.93 ± 0.44	6.82 ± 0.21
A Group	9.48 ± 0.28^{a}	9.21 ± 0.16^{a}	13.84 ± 0.66^{a}
B Group	8.41 ± 0.52^{a}	8.42 ± 0.21^{a}	13.21 ± 0.08^{a}
C Group	9.65 ± 0.23^{a}	7.97 ± 0.28^{a}	15.72 ± 1.76^{a,b,c}

Different letters express significative differences (P<0.05). $^{a, b, c}$: A statistically significant difference exists between the mean (±SE) values represented by different letters on the same column. (P<0.05). ^{a}: Control Group, ^{b}: A Group, ^{c}: B Group

TABLE III indicated the response of catalase, another important antioxidant enzyme, upon the administration of naproxen to *G. pulex*. The data show that there is a remarkable increase (P<0.05) in CAT activity in the whole body tissue of *G. pulex*, especially at 48 and 96 h after exposure compared to the control group. These changes were observed at a dose of 6.87 and13.75 mg·L^{-1} of naproxen, and it is remarkable (P<0.05) to note that no deaths were reported among *G. pulex* throughout the entire treatment duration at these doses.

TABLE III
Catalase levels (units·mg^{-1} protein·min^{-1}, mean ± standard deviation) in 24–96 hours naproxen exposed and controls *Gammarus pulex*

	24 hours	48 hours	96 hours
Control Group	4.90 ± 0.24	4.08 ± 0.32	4.64 ± 0.19
A Group	4.87 ± 0.33	4.31 ± 0.23	4.71 ± 0.12
B Group	5.67 ± 0.11^{a,b}	5.98 ± 0.25^{a,b}	6.43 ± 0.09^{a,b}
C Group	8.54 ± 0.34^{a,b,c}	8.37 ± 0.33^{a,b,c}	9.76 ± 0.73^{a,b,c}

Different letters express significative differences (P<0.05). $^{a, b, c}$: A statistically significant difference exists between the mean (±SE) values represented by different letters on the same column. (P<0.05). ^{a}: Control Group, ^{b}: A Group, ^{c}: B Group

The results presented of GSH in TABLE IV provide additional evidence supporting the presence of oxidative stress resulting from exposure to Naproxen. A remarkable decrease in GSH levels was observed in the whole body tissue of *G. pulex* in parallel with the dose increase in all groups compared to the control group. These findings strongly suggest the occurrence of oxidative stress in *G. pulex* following naproxen exposure. It is noteworthy that no deaths were observed in *G. pulex* at this dose throughout the treatment period. It is the crucial point that notable alterations were not observed in this parameter at lower doses (P>0.05).

TABLE IV
Superoxide dismutase activity (μmol·mg^{-1} protein, mean ± standard deviation) in 24–96 hours naproxen exposed and controls *Gammarus pulex*

	24 hours	48 hours	96 hours
Control Group	4.83 ± 0.43	5.47 ± 0.62	5.23 ± 0.48
A Group	4.91 ± 0.27^{a}	3.98 ± 0.33^{a}	3.44 ± 0.43^{a}
B Group	4.67 ± 0.23^{a}	3.41 ± 0.17^{a}	3.10 ± 0.17^{a}
C Group	4.11 ± 0.74^{a,b,c}	3.68 ± 0.21^{a}	2.54 ± 0.28^{a,c}

Different letters express significative differences (P<0.05). $^{a, b, c}$: A statistically significant difference exists between the mean (±SE) values represented by different letters on the same column. (P<0.05). ^{a}: Control Group, ^{b}: A Group, ^{c}: B Group

Antioxidant enzymes play a vital role in protecting cells by scavenging and inhibiting the formation and activity of harmful free radicals. They are essential for preventing tissue damage caused by chemical compounds. Industrial substances can affect the activities of these enzymes, either increasing or decreasing them, while some enzymes remain unaffected [38]. Numerous studies have investigated the impact of NSAIDs including naproxen on antioxidant enzymes in aquatic organisms [18, 19, 20, 21, 23, 39, 40, 41, 42]. Naproxen have been shown to induce oxidative stress and cellular degenerative processes by increasing of enzymes such as SOD and CAT activities within tissues during sublethal exposure [20, 43]. Lipid peroxidation is a process that necessitates the uptake of oxygen (O_2) and leads to the generation of superoxide radicals. Exposure of *G. pulex* to carvedilol may induce the production of superoxide dismutase and catalase, potentially resulting in the accumulation of O_2 and H_2O_2. This accumulation of O_2 and H_2O_2 could contribute to an elevation in lipid peroxidation levels in *G. pulex*. The increased lipid peroxidation observed in the experimental groups might be attributed to enhanced oxygen uptake and a more pronounced induction of SOD and CAT activity. Lipid peroxidation is recognized as one of the detrimental effects caused by reactive oxygen species. During this process, lipid peroxides readily decompose, leading to the release of highly reactive carbonyl fragments, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Tissue levels of MDA or the extent of lipid peroxidation are commonly assessed through the measurement of tissue thiobarbituric acid reactive substances (TBARS), which are indicative of tissue damage caused by free radicals [44]. Several studies [19, 20, 43, 45, 46] have reported increased MDA activity in fish and aquatic invertebrates exposed to NSAIDs. The level of MDA activity exhibited a remarkable increase (P<0.05) in tested whole body tissue in a time-dependent manner after naproxen treatment of *G. pulex*.

Superoxide dismutase (SOD) enzymes play a crucial role in neutralizing superoxide radicals and can counteract lipid peroxidation induced by oxidative stress. In the present study, naproxen administration resulted in the increase in SOD activity. The changes in SOD activity observed in tissues of aquatic animals after naproxen and other NSAIDs administration align with the findings of various other researchers [23, 47, 48, 49]. Lucero *et al.* [21] investigated the

SOD activity in the *Hyalella azteca* using a naproxen concentration of 76.6 and 339.2 mg·kg^{-1} and reported a increase in SOD content following naproxen administration in the *Daphnia manga* [20]. The present studies' results are consistent with the findings of the above mentioned authors. However, several studies [48, 50] have reported an decrease in SOD activity in *D. magna* and *Rhamdia quelen* when exposed to NSAIDs such as ibuprofen and diclofenac. For example, Cikcikoglu Yıldirim *et al*. [23] reported a remarkable decrease followed by a decrease in SOD activity in *G. pulex* exposed to ibuprofen and propranolol. These discrepancies may be attributed to variations in animal species and the administration method of NSAIDs in the respective studies. A notable rise in superoxide dismutase (SOD) activity was evident at 24 and 48 h (P<0.01). However, no remarkable differences were observed between the treatment groups and the control group after 48 h (P>0.05). This pattern aligns with the role of SOD as the primary defense against reactive oxygen species. SOD functions by converting superoxide radicals into oxygen molecules or hydrogen peroxide [51]. The observed increase in catalase (CAT) activity suggests that SOD activity could possibly increase leading to higher hydrogen peroxide production, which was subsequently detoxified by CAT.

This study demonstrated that the average catalase (CAT) activity in *G. pulex* exhibited a significant increase (P<0.05), particularly at the 96th h. The increase in CAT activity was measured at 2.59, 11.18, and 2.16% respectively, in the respective experimental groups at the 96th h. Similar remarkable increases in CAT activity were observed by Zivna *et al*. [52] in *Danio rerio* exposed to acetylsalicylic acid, and by Cikcikoglu Yildirim *et al*. [53] in *G. pulex* exposed to various concentrations of ibuprofen over 24 and 96 h. Costa *et al*. [54] and Parolini *et al*. [18] Pawłowska *et al*. [55] described an increase in CAT activity in the *R. philippinarum* and *Ruditapes decussatus, D. polymorpha* and *Heterocypris incongruens* following administration of NSAIDs such as diclofenac, ibuprofen and naproxen. However, it could be seen that there are studies reporting conflicting findings. Nunes *et al*. [56] found that diclofenac had no effect on CAT activity in *Solea senegalensis*. Similar observations were reported in *Cyprinus carpio* exposed to acetylsalicylic acid [52]. In this study, a dose-dependent increase in CAT activity was observed. In *Scrobicularia plana*, ibuprofen exposure produced biochemical alterations (SOD, CAT, lipid peroxidation) in several organs indicating oxidative stress and damage to lipids [57].

In the current investigation, there was a remarkable decline observed in the concentration of GSH within the treatment groups, compared to the control group. This finding substantiates the occurrence of cellular oxidation and implies an inadequate functioning of the glutathione system in effectively clearing ROS generated by naproxen. In results are in line with previous study, where naproxen administration decreased the level of GSH compared to the experimental and control groups in Wistar rats [58]. In the study, naproxen administration resulted in the increase of superoxide dismutase (SOD) activity, leading to higher levels of H_2O_2, which may have been mitigated by the action of catalase (CAT), which was also bioactivated. The decrease in GSH content could be attributed to the elimination of H_2O_2 by GSH under conditions of stress. Additionally, there is a strong and significant correlation between MDA and glutathione levels, indicating that higher MDA levels correspond to lower glutathione levels. There is a strong and significant correlation between MDA and glutathione levels; This indicates that higher MDA levels correspond to lower glutathione levels [59].

CONCLUSION

The findings of this study demonstrated the capacity of naproxen to induce oxidative stress in G. pulex and increase MDA levels even at extremely low concentrations. This situation; Since it will negatively affect natural water resources and the creatures living there, it should be considered as an environmental risk and effective methodologies should be developed to prevent naproxen from entering the aquatic environment. Heavy metals, pesticides, organic pollutants, ammonia and nitrates, and drug residues can cause harmful effects on aquatic creatures such as G. pulex. Examining the effects of these substances on G. pulex can provide important data for the health of the ecosystem and conservation efforts. This study provides a more comprehensive understanding of how toxic substances affect the physiology of *G. pulex*. One possible mechanism of action of these toxic materials is their ability to modify the characteristics of lipid peroxidation constituents and the antioxidant status in aquatic invertebrates. The alterations observed in antioxidant enzymes suggest that an adaptive stress response may be triggered in organisms exposed to naproxen, aiming to restore the redox balance and prevent oxidative damage.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or vertebrate animals were used in the present research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

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