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Toxicity of pharmaceutical micropollutants on common carp (*Cyprinus carpio*) using blood biomarkers

Walaa M. Shaalan[1,2] & Alaa El-Din H. Sayed[3,4✉]

The presence of pharmaceutical compounds in aquatic environments has become a notable ecological issue, with compounds such as bromazepam, naproxen, metoprolol, and sotalol being of particular concern due to their prevalence and potential biological effects on non-target species. This study aims to evaluate the effects of these pharmaceuticals on common carp (*Cyprinus carpio*), focusing on cytogenicity, genotoxicity, hematological, biochemical, neurological, and immunological responses. Juvenile carp were exposed to 15.54 µg/L bromazepam, 14.40 µg/L naproxen, 5.76 µg/L metoprolol, and 3.33 µg/L sotalol, respectively. Hematological analysis revealed significant reductions in red blood cell counts and hemoglobin levels, indicating potential anemia, especially in fish exposed to naproxen. Biochemical assays showed increased levels of albumin, globulin, and enzymes indicative of liver stress in exposed groups. Antioxidant defense enzymes such as superoxide dismutase, catalase, and glutathione peroxidase showed significant activity increases, suggesting oxidative stress. Apoptosis assays demonstrated elevated erythrocyte apoptosis, particularly in the naproxen group, which also exhibited the highest DNA damage. These findings highlight the ecological significance of pharmaceutical contamination and highlight the urgent need for thorough environmental risk assessments that take into account the health of both organisms and ecosystems, as well as the creation of mitigation plans for their detrimental effects on aquatic ecosystems.

Keywords Aquatic environment, Apoptosis comet, Superoxide dismutase, Catalase, *Cyprinus carpio*, Genotoxicity, LDH

In recent years, the presence of pharmaceutical compounds in aquatic environments has emerged as a significant ecological concern[1]. These compounds originated from various sources such as wastewater effluents, agricultural runoff, and improper disposal. They have been detected in surface waters globally at concentrations that, while often low, pose potential risks to aquatic organisms[2]. Pharmaceuticals are designed to have biological effects at low doses, raising concerns about their unintended impacts on non-target species when they enter the environment[3]. Among the multitude of pharmaceuticals found in aquatic environments, bromazepam, naproxen, metoprolol, and sotalol are particularly noteworthy due to their widespread use and documented presence in water bodies[4–7]. Bromazepam, a benzodiazepine, is commonly prescribed for its anxiolytic properties[8]. Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) used to treat pain and inflammation[9]. Metoprolol and sotalol are beta-blockers widely used in the treatment of cardiovascular diseases[10,11]. The extensive use of these pharmaceuticals leads to their persistent presence in the environment, where they can exert various biological effects on aquatic organisms[12].

For aquatic animals, exposure to pharmaceutically active compounds (PhACs) in the environment may have significant sub-lethal ecological effects[13]. In particular, psychoactive medications are meant to modify behavior in humans as part of the prescribed treatment (antidepressants, anxiolytics, antipsychotics), or as a side effect of some medications (analgesics). They may also have a similar effect on aquatic life. This is due to the fact that many pharmacological targets and receptors are extensively conserved throughout the vertebrate taxonomic tree; so, fish may exhibit effects that are comparable to those observed in humans[14]. Pharmaceuticals including naproxen have reportedly been found in the plasma of fish kept in aquariums exposed to treated wastewater[15].

[1]Zoology Department, Faculty of Science, Benha University, Benha 13518, Egypt. [2]Bioinformatics Group, Faculty for Biology and Biotechnology and Center for Protein Diagnostics, Ruhr-University Bochum, 44801 Bochum, Germany. [3]Department of Zoology, Faculty of Science, Assiut University, Assiut 71516, Egypt. [4]Molecular Biology Research and Studies Institute, Assiut University, Assiut 71516, Egypt. ✉email: alaasayed@aun.edu.eg

Bromazepam in the aquatic environment leads to some behavioral changes in the aquatic organisms[16]. It can cause sedation and reduce the overall activity levels of fish[17]. This can affect their ability to forage, evade predators, and interact with their environment[18]. Fish exposed to bromazepam may exhibit changes in social behavior, including reduced aggression and altered schooling behavior. This can disrupt social hierarchies and breeding patterns[18]. Bromazepam is reported to have physiological impact on aquatic fish. Bromazepam exposure can interfere with the normal stress response in fish, making them less able to cope with environmental stressors[19].

Juvenile meagre fish (*Argyrosomus regius*) exposed to venlafaxine, an antidepressant with pharmacological effects similar to those of bromazepam, may experience decreased reproductive success, either through direct effects on reproductive organs or through behavioral changes that affect mating[20]. *Danio rerio* can be beneficial when evaluating the toxicological effects of drugs that may have antidepressant properties[21]. As pollutants, antidepressant medications cause an immense amount of pollution in the environment. As a result of their mode of action, which involves modulating neurotransmitter activity, it is hypothesized that they may affect various biological processes in living things exposed to their residues in the water system by interfering with their development, behavior, and reproduction[22]. When sertraline was tested at low concentrations in the environment, it was found to accelerate the hatching process of *Danio rerio* larvae and induce altered behavior, such as avoiding the dark zones of a tank[23]. Similarly, zebrafish larvae exhibited hypolocomotion and delayed hatching when exposed to fluoxetine at levels found in municipal water samples. Additionally, the tested subjects' acetylcholinesterase (AChE) activity was shown to be inhibited in a dose-dependent manner[24]. However[21], showed that venlafaxine concentrations in the environment had two different effects on *Danio rerio* larvae activity: at higher tested concentrations, it decreased zebrafish mobility, while at lower tested concentrations, it induced hyperlocomotion. Embryos and larvae exposed to antidepressant might experience developmental abnormalities, potentially leading to higher mortality rates in early life stages[21]. Subchronic exposure to naproxen was reported to have significant effects on the early life stages of common carp. These effects included disturbances in hatching, developmental rate, morphology, and histology, as well as increased mortality when combined with tramadol hydrochloride[25]. Previous studies subjected rainbow trout (*Oncorhynchus mykiss*) to low doses of naproxen in aquaria[26]. They observed that the metabolites and total bioconcentration factors of naproxen in the bile of fish exposed to water varied from 500 to 2,300. It was reported that zebrafish exposed to metoprolol exhibited a reduced heart rate[27]. The hatching rate of fish embryos decreases with increasing exposure to metoprolol[27]. Additionally, there was a tendency for the metoprolol exposure to slightly raise fish mortality[28]. Previous study has been done on sotalol and its effect on the New Zealand mudsnail *Potamopyrgus antipodarum*[29]. The authors postulated that there would be fewer embryos in the brood pouches, slower adult snail growth, smaller newborns, and no influence on adult death rates based on the pharmacodynamic features of sotalol. The acute immobilization test with *Daphnia magna* indicated that the median lethal concentration was substantially higher than environmental concentrations[30].

Common carp was selected for this study due to its ecological and scientific relevance. Common carp is a species with global distribution and significant importance in aquaculture, making it an ideal model organism for assessing environmental toxicity. Additionally, it is listed in the OECD guidelines as a recommended species for chemical testing[31]. Moreover, we specifically chose juvenile carp to avoid gender-related influences and because younger life stages are generally more sensitive to chemical exposures than adults. This allows us to better assess the potential sub-lethal effects of pharmaceutical exposure in an environmentally and scientifically relevant species. The selection of bromazepam, naproxen, metoprolol, and sotalol for this study was driven by their widespread use, environmental persistence, and distinct pharmacological profiles that target different biological systems, making them representative of diverse pharmaceutical classes commonly detected in aquatic environments. Bromazepam, a psychoactive benzodiazepine, was included due to its potential to induce behavioral and stress-related changes in aquatic organisms, as evidenced by its effects on social interactions and coping mechanisms[32]. Naproxen, a widely used NSAID, was chosen because NSAIDs are among the most frequently detected pharmaceutical contaminants globally and have documented impacts on aquatic species, including developmental and reproductive effects[33]. Metoprolol, a beta-blocker, was selected for its known effects on cardiac function, hatching, and survival rates in fish embryos, while sotalol, another beta-blocker, was included due to its documented influence on growth and reproduction in aquatic invertebrates[34]. Together, these pharmaceuticals represent a range of commonly used drugs with varying mechanisms of action, providing valuable insights into their ecological impacts and serving as a basis for assessing the broader environmental risks of pharmaceutical mixtures. Current environmental risk assessment methodologies emphasize the importance of understanding the persistence, bioaccumulation potential, and toxicity profiles of individual contaminants to evaluate their ecological impact. However, aquatic organisms are rarely exposed to single compounds in isolation; instead, they encounter complex mixtures of various pharmaceuticals and other pollutants, which can interact in additive, synergistic, or antagonistic ways. The concentrations of bromazepam, naproxen, metoprolol, and sotalol used in this study were selected based on their reported maximum concentrations in effluent from wastewater treatment plants, as indicated in recent literature[35]. These concentrations represent levels of pharmaceutical contaminants that are typically found in treated wastewater and serve as a realistic representation of environmental exposure. By using these concentrations, our study aims to evaluate the potential ecological effects of these pharmaceuticals under conditions that closely mirror actual environmental contamination, ensuring that our findings are both relevant and applicable to real-world scenarios. Studies focused on individual pharmaceuticals are crucial initial steps for determining baseline toxicity and understanding specific modes of action, as these insights help to predict potential risks in multi-contaminant scenarios. This study aims to evaluate the effects of bromazepam, naproxen, metoprolol, and sotalol on common carp (*Cyprinus carpio*), focusing on cytogenicity and genotoxicity, hematological and biochemical parameters, and neurological and immunological responses.

Materials and methods

The current study followed the guidelines of the Animal Use and Care Committee of Assiut University, Egypt (Experimental protocol Number 07\2024\0056).

Chemicals

Bromazepam (CAS Number:1812-30-2), Naproxen CAS No.:22204-53-1), metoprolol (CAS No.:56392-17-7), and sotalol (CAS No.:959-24-0) were purchased from Sigma Aldrich (Deisenhofen, Germany).

Experimental set up and sampling procedures

The juvenile common carp (*Cyprinus carpio* L.) was purchased from Aquaponic unit at Assiut University, Egypt. The acclimation of the test organisms was carried out over a period of two weeks to ensure they adjusted to the experimental conditions. Following acclimation, the experiment was conducted in rectangular tanks, each with dimensions of 100 cm × 70 cm × 50 cm. These tanks were used to house the organisms and allowed for continuous monitoring of key physicochemical parameters of the water throughout the study. The water in the tanks was maintained at an average temperature of 28.5 ± 0.21°C, with a pH value of 7.4 ± 0.35, providing a stable and neutral environment. Dissolved oxygen (DO) levels were kept at 6.9 ± 0.56 mg/L to ensure adequate oxygenation for the organisms, and the conductivity of the water was measured at 260.8 ± 5.26 mS/cm, reflecting the ionic composition and salinity. To maintain water quality and reduce waste products generated by the fish, 40% of the water volume in each tank was replaced daily. The replaced water was carefully re-dosed with appropriate treatments to match the original conditions, ensuring minimal disruption to the experimental parameters. The tanks were also subjected to a controlled photoperiod, set to a 12-h light: 12-h dark cycle (12:12). This simulated natural environmental conditions and ensured a consistent day-night rhythm for the duration of the experiment. Common carp fish with an average weight of 40.5 ± 6.74 g were divided into five groups with 30 fish per group. The experiment was done in triplicate tanks per group for two weeks. Four groups were exposed to bromazepam, naproxen, metoprolol, and sotalol at nominal concentrations of 15.54 μg/L, 14.40 μg/L, 5.76 μg/L, and 3.33 μg/L, respectively[35], while one group served as the control. The drugs were dissolved in distilled water to prepare the desired nominal concentrations and were added directly to the experimental tanks during daily water replacement. As distilled water was the solvent used, no separate vehicle control group was included, and the control group consisted of fish exposed to the same environmental conditions without any added drugs. At the end time, six fish from each group were chosen at random for sampling. Fish were placed on ice to minimize stress[36,37].

Hematological and biochemical analysis

Blood samples were collected from the caudal veins of six fish per group and immediately placed in heparinized tubes. The Hb concentration and the number of blood cells were estimated within 30 min of blood collection. An automated technical analyzer (Mindray Bc-2800) was used to measure the red blood cells (RBCs, Million/μl), white blood cells (WBCs, Thousands/μl), hematocrit (Ht, %), and hemoglobin (Hb, g/dl). The formulae described by[38] were used to compute the mean corpuscular volume (MCV, μm³), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, %). After being diluted 1:20 with a diluting fluid (saline solution), blood was put into a hemocytometer. The hemocytometer's four large (1 mm²) corner squares were counted under an Olympus microscope at 640×. The total number of WBCs was computed in mm³ × 10³, and the differential count (lymphocytes %, monocytes %, and neutrophils %) was demonstrated by staining blood films with Giemsa dye[39].

Serum biochemical parameters, including albumin, globulin, albumin/globulin (A/G) ratio, total protein, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), and lactate dehydrogenase (LDH), were measured using commercially available diagnostic assay kits specifically designed for serum/plasma samples. All kits were obtained from Spectrum Diagnostics (Egyptian Company for Biotechnology, Cairo, Egypt) except albumin kit from Genesis company, Egypt, and were used strictly according to the manufacturer's instructions without modification, unless otherwise specified. Each analyte's kit catalog number is reported below and confirmed to be traceable to actual commercial assays, per reviewer request. All measurements were conducted in duplicate. Serum samples were collected without anticoagulants and centrifuged at 3000×*g* for 10 min at 4 °C. Supernatants were stored at − 20 °C and thawed only once prior to analysis. No matrix-specific dilution or pretreatment was performed beyond kit instructions, unless noted.

Albumin (g/dL) was determined using a colorimetric bromocresol green (BCG) method (Cat. No. 111003). In brief, 10 μL of serum was mixed with 1.0 mL of BCG reagent (pH 4.3 citrate buffer) and incubated for 5 min at room temperature. The resulting green complex was measured at 578 nm using a Jasco-V530 spectrophotometer (Jasco, Ottawa, Canada). Albumin concentrations were calculated based on a provided standard curve[40]. Globulin (g/dL) was calculated by subtracting albumin from total protein values (Cat. No. 819002), obtained as described below. The A/G ratio was then derived by dividing albumin by globulin[41]. Total protein (g/dL) was measured using the Biuret method (Cat. No. 310001). A total of 20 μL of serum was mixed with 1.0 mL of Biuret reagent and incubated at room temperature (15–25 °C) for 10 min. Absorbance was read at 546 nm, and concentrations were calculated against a provided protein standard[42].

Glucose (mg/dL) was measured using the glucose oxidase–peroxidase (GOD-PAP) method (Cat. No. 250001). A 10 μL aliquot of serum was added to 1.0 mL of reagent and incubated for 10 min at 37 °C. Absorbance was recorded at 546 nm, and concentrations were calculated against the kit's 100 mg/dL glucose standard[43].

AST (U/L) activity was assessed using the 2,4-dinitrophenylhydrazine method (Cat. No. 260001). Briefly, 100 μL of serum was mixed with 0.5 mL of buffer reagent (R1) and incubated at 37 °C for 30 min. Then, 0.5 mL of color reagent (R2) was added and incubated at room temperature for 20 min, followed by addition of 5.0 mL

of 0.4 mol/L NaOH. Absorbance was measured at 546 nm[44]. ALT (U/L) activity was determined using the same hydrazone-based principle (Cat. No. 264001). The assay followed the same steps as AST, using ALT-specific buffer and reagents[45]. ALP (U/L) was quantified using a kinetic colorimetric assay based on the IFCC method (Cat. No. 217003). Serum (10 µL) was added to 1.0 mL of reagent containing p-nitrophenyl phosphate in alkaline buffer. The reaction was monitored at 405 nm every 60 s for 3 min after a 180-s delay at 37 °C. Enzyme activity was calculated using the kit's kinetic formula[46].

Creatine kinase (CK; U/L) was measured using a kinetic enzymatic method aligned with DGKC and IFCC recommendations (Cat. No. 238004). A working reagent was prepared by mixing four parts of Reagent 1 with one part of Reagent 2. A 20 µL serum sample was added to 0.5 mL of this working reagent, and absorbance was monitored at 340 nm at 1-min intervals for 3 min at 37 °C. CK activity was calculated from the NADPH formation rate[47].

Lactate dehydrogenase (LDH; U/L) was assessed via a UV kinetic assay (Cat. No. 283005). The working reagent (phosphate buffer with pyruvate and NADH) was freshly prepared by mixing 4 parts Reagent 1 and 1 part Reagent 2. In each assay, 500 µL of the working reagent was added to 10 µL of serum in a pre-warmed cuvette (37 °C). Absorbance was recorded at 340 nm at 1-min intervals for 3 min. LDH activity was calculated based on the rate of NADH oxidation[48].

All biochemical assays were validated for linearity, reproducibility, and were performed in accordance with the kit's stated intended matrix (serum). The same batch of reagents was used across experimental groups to minimize inter-assay variability.

Antioxidant parameters measurements

Superoxide Dismutase (SOD, Unit/mL), Catalase (CAT, Unit/mL), and Glutathione Peroxidase (GPX) activities were measured using kits of SGMitalia Company USA. Each sample was tested in triplicate using a Biochrom Ltd microplate reader to measure the SOD levels in serum at 450 nm[49,50]. The CAT activity in serum was measured in triplicate using a UV–VIS spectrophotometer set to 240 nm[49]. GPX activity was determined following the method described by[51].

Apoptosis and comet assay

According to[52], methanol-fixed smears stained with Acridine Orange dye (Life Technologies, Carlsbad, USA) were used to identify apoptotic erythrocytes. Cells were examined using a Zeiss Axioplan 2 fluorescent microscope (×200) equipped with a Sony AVT-Horn digital 3 CCD color video camera. According to[53], blood (50 µL) was drawn from the caudal veins after two weeks of exposure. It was then promptly frozen to prevent endogenous DNA damage and to prevent DNA repair in the unfixed cells[54]. A hemocytometer was also used to evaluate cell viability. A previously published procedure by[55] was used for a neutral comet test, with a few minor adjustments[53]. A Zeiss Axioplan 2 fluorescent microscope (×200) equipped with a digital 3 CCD color video camera (Sony, AVT-Horn) was used to observe the cells. The tail moment score was computed by analyzing each cell's comet picture using CASP software[56].

Statistical analysis

All data were reported as mean ± standard error of the mean (SEM) using statistical analysis performed using GraphPad Prism 9.0 (GraphPad Software Inc.). One-way ANOVA and post-hoc Tukey's HSD tests were conducted to identify significant differences between all tested groups and from controls as well as between each other. The statistical significance of differences between treatments and controls was set at $P \leq 0.05$. Asterisk superscript (*) visually indicated the significant level (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Results
Hematological parameters

Hematological testing in fish is essential for assessing health status and detecting metabolic diseases by measuring blood parameters and comparing them to established norms, as shown in Table 1. In this study, fish exposed to bromazepam, naproxen, metoprolol, and sotalol exhibited significant reductions in red blood cell (RBC) counts compared to the control group (1.392 ± 0.038 million/µL). The RBC counts for the treated groups were recorded as follows: bromazepam (1.3 ± 0.018 million/µL), naproxen (1.225 ± 0.030 million/µL), metoprolol (1.265 ± 0.018 million/µL), and sotalol (1.313 ± 0.039 million/µL). Significant differences ($p \leq 0.05$) were observed between the bromazepam and naproxen groups, as well as between the naproxen and sotalol groups (Fig. 1a). Hemoglobin levels were significantly reduced ($p \leq 0.05$) in the bromazepam, naproxen, and metoprolol groups compared to controls, while the sotalol group exhibited a non-significant decrease. However, hemoglobin levels in the sotalol group were significantly higher ($p \leq 0.05$) than in the naproxen group. Additionally, hemoglobin levels in the bromazepam and metoprolol groups were significantly higher ($p \leq 0.05$) than in the naproxen group (Fig. 1b). Hematocrit (Ht) levels in the naproxen group were significantly lower ($p \leq 0.05$) than in all other tested groups, including the control group (Fig. 1c). Mean corpuscular volume (MCV) showed a significant increase in the bromazepam, metoprolol, and sotalol groups relative to controls (Fig. 1d). Mean corpuscular hemoglobin (MCH) was significantly increased ($p \leq 0.05$) only in the sotalol group, with no significant changes observed in the other groups (Fig. 1e). MCHC was non-significantly changes in tested groups compared to the control group (Fig. 1f). White blood cell (WBC) counts were significantly reduced ($p \leq 0.05$) in the bromazepam and naproxen groups compared to controls, with significant variations recorded among the tested groups (Fig. 1). Neutrophil counts did not differ significantly between the tested groups and the control, but significant differences were observed among the tested groups themselves. Lymphocyte counts were significantly higher in the sotalol group compared to the control group, while monocyte counts were decreased across all tested groups compared to controls.

	Control	Bromazepam	Naproxen	Metoprolol	Sotalol
RBcs (Million/ μL)	1.392 ± 0.038	1.3* ± 0.018	1.225* ± 0.030	1.265* ± 0.018	1.313 ± 0.039
Hb (g/dL)	9.097 ± 0.196	8.613* ± 0.128	7.920* ± 0.395	8.668* ± 0.015	8.963 ± 0.179
Ht (PCV) %	37.280 ± 0.554	37.020 ± 0.691	33.790* ± 1.016	35.99 ± 0.666	37.170 ± 1.065
MCV (μm³)	254.900 ± 8.799	270.200* ± 6.212	262.400 ± 9.192	269.600* ± 7.084	278.600* ± 8.585
MCH (Pg)	62.180 ± 1.383	62.880 ± 1.132	61.550 ± 4.421	64.970 ± 0.845	67.130* ± 1.201
MCHC (%)	23.180 ± 0.475	22.120 ± 0.412	22.300 ± 1.468	22.920 ± 0.458	23.670 ± 0.671
WBcs (Thousands/μL)	51.020 ± 0.768	49.120* ± 0.768	47.380* ± 1.277	49.52 ± 0.9786	50.730 ± 1.108
Neutrophils (%)	15.670 ± 0.817	14.670 ± 0.817	16.500 ± 0.548	16.500 ± 0.548	14.830 ± 1.169
Lymphocytes (%)	81.330 ± 0.817	82.830 ± 1.169	81.500 ± 0.548	81.500 ± 0.548	83.330* ± 1.211
Monocytes (%)	3 ± 0	2.5 ± 0.5477	2* ± 0.6325	2* ± 0	1.833* ± 0.408

Table 1. Effects of pharmaceutical micropollutants on Common carp (*Cyprinus carpio*) exposure on the hematological biomarkers. Values are presented as mean ± standard deviation. Asterisks (*) indicate significant differences compared to the control group ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's post hoc test.

Biochemical parameters

The hepatopancreatic function parameters affected by exposure to bromazepam, naproxen, metoprolol, and sotalol were presented in Table 2. Albumin increased in all tested groups more than the control group. It increased significantly ($p \leq 0.05$) in fish groups exposed to naproxen and sotalol compared to the control group (Fig. 2a). Globulin changed in all tested groups compared to the control group. In addition, it was significantly increased ($p \leq 0.05$) in the naproxen group compared to the control group. A/G ratio was not significantly changed in all groups relative to the control one (Fig. 2b, c). Total protein, glucose, AlP, and lactate dehydrogenase were significantly increased ($p \leq 0.05$) in all groups compared to the control fish (Fig. 2d, e, g, h, j). AST and creatine kinase were significantly increased ($p \leq 0.05$) in fish groups exposed to bromazepam, naproxen, and sotalol compared to the control group (Fig. 2f, i).

Antioxidant defense enzymes

Table 3 illustrates how the antioxidant defense enzymes of common carp are affected by bromazepam, naproxen, metoprolol, and sotalol. In comparison to the control group, there was a significant increase in superoxide dismutase SOD ($P \leq 0.05$) following exposure to bromazepam, naproxen, metoprolol, and sotalol (Fig. 3b). Furthermore, compared to the control group, there was a substantial increase in catalase ($P \leq 0.05$) following exposure to bromazepam, naproxen, metoprolol, and sotalol (Fig. 3a). Furthermore, after being exposed to sotalol, naproxen, metoprolol, and bromazepam, GPx increased significantly ($P < 0.0001$) in comparison to the control group (Fig. 3C).

Apoptosis level and DNA damage

In comparison to the control group, the percentage of apoptotic erythrocytes in common carp exposed to bromazepam increased significantly at ($p \leq 0.05$). Exposure to naproxen leads to a significant increase in the percentage of apoptosis compared to the control group and other treated groups ($p \leq 0.05$). Fish groups exposed to metoprolol and sotalol showed markedly increased apoptosis relative to the control group as presented in Fig. 4a.

The amount of DNA damage was evaluated by measuring the percentage tail moment. Figure 4b displays the comet assay tail moment values. After exposure to naproxen, metoprolol, and sotalol, fish groups showed a distinct and statistically significant increase in tail moment values compared to the control group ($p \leq 0.05$). The naproxen exposure showed the highest amount of DNA damage.

Discussion
Hemato-biochemical parameters

Hematological testing is a vital tool for assessing the health and metabolic status of fish by providing detailed information on blood parameters, which can indicate underlying physiological and pathological conditions[57–59]. Numerous publications claim that hazardous substances can cause erythrocyte morphological abnormalities such amitosis, hemolysis, distortion of the cells, or nuclear abnormalities[60,61]. In this study, we observed significant alterations in several hematological parameters in fish exposed to bromazepam, naproxen, metoprolol, and sotalol compared to the control group, indicating the potential impact of these substances on fish health. The significant reduction in red blood cell (RBCs) counts in the treated groups highlights a potential hematotoxic effect of these substances. While these effects are observed in carp at environmentally relevant concentrations, it is important to note that therapeutic doses in humans are significantly higher (e.g., sotalol is prescribed at 80–320 mg/day in humans[62], compared to 3.33 μg/L in this study). Regulatory documents from the FDA and EMA do not report similar hematotoxic effects (e.g., anemia, RBC suppression) in humans at therapeutic doses, suggesting species-specific differences in susceptibility. Carp may be more vulnerable due to differences in metabolism, detoxification capacity, and continuous exposure routes[63]. The specific decrease in RBCs could be indicative of anemia or impaired erythropoiesis[64], which might be caused by the direct or indirect toxic effects
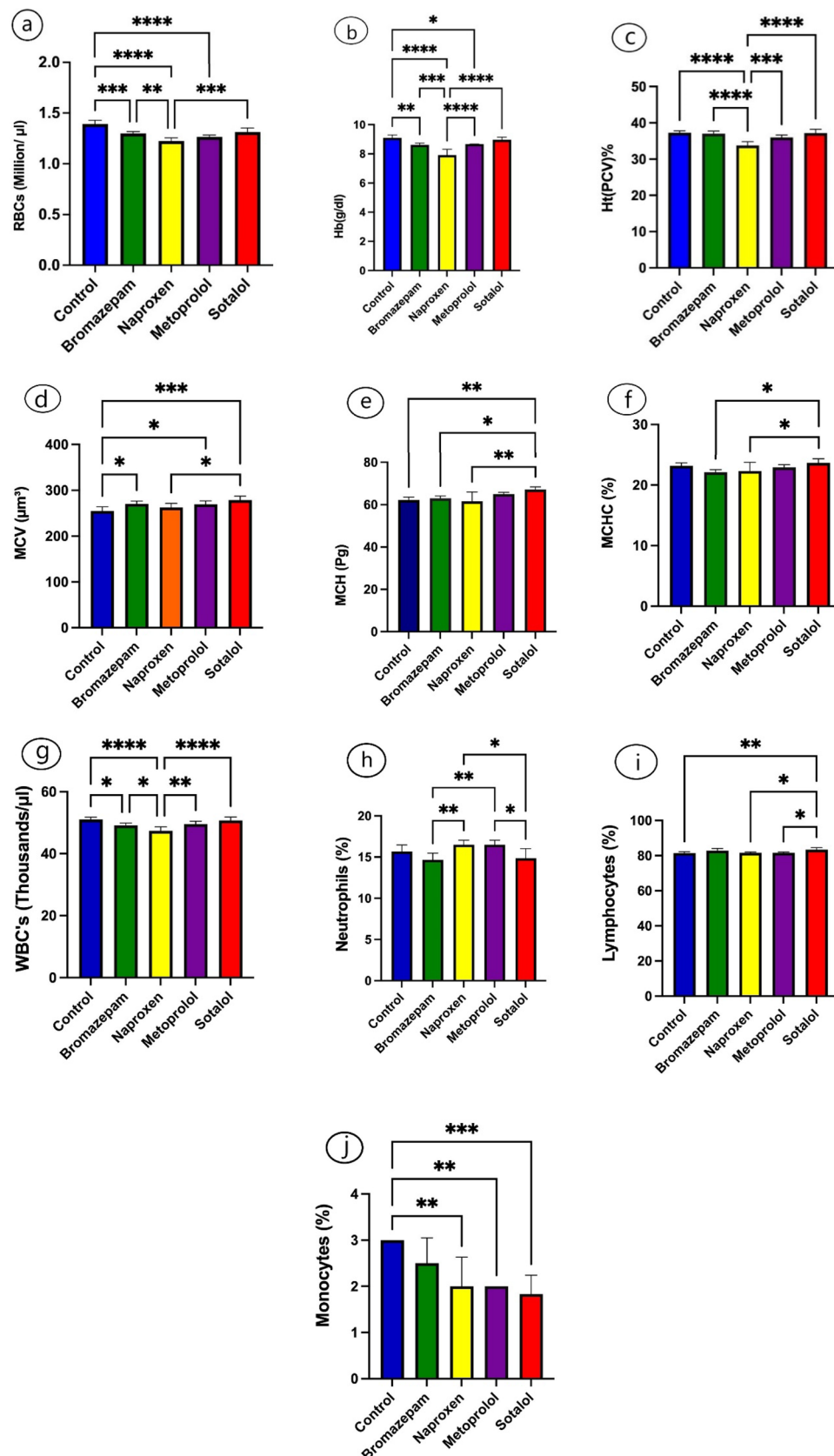
Fig. 1. The effects of bromazepam, naproxen, metoprolol, and sotalol on common carp hemocyte counts. The mean ± SE is used to present the data (n = 6). Asterisks indicate significant differences from the control group (*P* < 0.05, *; *P* < 0.01, **; *P* < 0.001, ***; and *P* < 0.0001, ****).

	Control	Bromazepam	Naproxen	Metoprolol	Sotalol
Albumin (g/dL)	0.703 ± 0.022	0.728 ± 0.013	0.77* ± 0.017	0.715 ± 0.015	0.738* ± 0.027
Globulin (g/dL)	2.037 ± 0.025	2.123 ± 0.074	2.287* ± 0.046	2.035 ± 0.038	2.107 ± 0.061
A/G ratio	0.325 ± 0.013	0.3248 ± 0.009	0.3168 ± 0.003	0.329 ± 0.009	0.340 ± 0.012
total protein (g/dL)	2.590 ± 0.092	2.787* ± 0.043	2.795* ± 0.038	2.740* ± 0.043	2.830* ± 0.086
Glucose (mg/dL)	52.970 ± 0.873	55.23* ± 0.826	55.77* ± 0.441	54.550 ± 1.157	56.330* ± 2.160
AST (U/L)	78.700 ± 0.957	81 ± 0.696	82* ± 0.863	81.470* ± 1.164	84.180* ± 2.52
ALT (U/L)	46 ± 1.115	48.670* ± 0.720	50.38* ± 1.641	48.830* ± 0.817	50.480* ± 1.667
LP(U/L)	7.777 ± 0.045	8.160* ± 0.094	8.423* ± 0.1067	8.108* ± 0.112	8.398* ± 0.279
Creatine kinase (U/L)	78.72 ± 0.983	82.050* ± 1.060	82.42* ± 0.697	81.03 ± 1.551	83.730* ± 2.532
Lactate dehydrogenase (U/L)	70.38* ± 1.534	75.830* ± 3.676	82.8* ± 1.765	74.700* ± 0.959	77.170* ± 1.323

Table 2. Effects of pharmaceutical micropollutants on Common carp (*Cyprinus carpio*) exposure on the biochemical biomarkers. Values are presented as mean ± standard deviation. Asterisks (*) indicate significant differences compared to the control group ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's post hoc test.

of these compounds on the hematopoietic system. It was reported that many fish species are susceptible to poikilocytosis, which can be triggered by a variety of factors including pollution exposure, nutritional deficits, pathogenic agents, and environmental stressors[65,66]. Some pharmaceuticals may cause oxidative stress, which can result in hemolysis, or the deterioration of red blood cells. An imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense mechanisms leads to oxidative stress. This may harm red blood cell membranes, rupturing them and lowering the number of red blood cells[67]. Certain substances might be harmful to the bone marrow, the hematopoietic tissue that produces red blood cells, which would slow down the process of making red blood cells, or erythropoiesis[68].

Furthermore, hemoglobin levels were significantly reduced in the bromazepam, naproxen, and metoprolol groups. This reduction in hemoglobin could be a result of decreased RBCs counts, suggesting that the oxygen-carrying capacity of the blood is compromised in these fish[69]. Interestingly, hemoglobin levels were significantly higher in the sotalol group compared to the naproxen group, which may indicate different mechanisms of action or varying degrees of hematological impact among the tested compounds. Previous work on naproxen reported that the hematological results exhibited a significant decrease in WBC, RBC, hemoglobin, and hematocrit in the group treated with naproxen compared to the control group[70]. They reported that naproxen treatment led to a significant decrease in red blood cell (RBC) count. This decrease is thought to occur due to the production of free radicals, which interact with the cell membranes of RBCs, resulting in hemolysis and the destruction of red blood cells[71]. Additionally, naproxen has been shown to reduce hemoglobin levels, a finding that aligns with previous work suggesting that the destruction of RBCs is a contributing factor to this effect[72]. Furthermore, naproxen's impact on hematocrit levels could be linked to its influence on platelet aggregation. Naproxen has been shown to impair platelet aggregation, which could explain the decreased hematocrit observed in our study, as supported by findings in human studies[73].

The hematocrit (Ht), which represents the percentage of red blood cells (RBCs) in relation to total blood volume, is an important hematological parameter that can provide insights into the oxygen-carrying capacity of the blood and overall blood health[74]. Changes in hematocrit levels can be indicative of various pathological conditions, including anemia, blood dilution, and dehydration, as well as alterations in RBC production or destruction. In our study, hematocrit (Ht) levels were significantly lower in the naproxen group compared to all other groups, including the control. This decrease further supports the evidence of anemia or blood dilution effects in the naproxen-treated fish. Mean corpuscular volume (MCV) was significantly increased in the bromazepam, metoprolol, and sotalol groups, which could suggest a compensatory response to maintain adequate oxygen transport by increasing the size of the remaining erythrocytes. Over the period of the 12-day exposure to anti-inflammatory drug, fish treated with acetylsalicylic acid exhibited a significant ($P < 0.05$) decrease in their levels of Hb, hemoglobin-ct, and RBCs in comparison to the control group. Conversely, there was a noteworthy ($P < 0.05$) rise in the MCV, MCH, MCHC, and WBC[75]. It was reported that exposure to chemical compounds (MeHg) may potentially impact the red blood cell turnover process, leading to an increase in the number of older cells in circulation and the induction of anemia[76]. The increase in MCH suggests that these compounds might affect hemoglobin synthesis or erythrocyte morphology, potentially leading to a higher hemoglobin content per cell without a proportional increase in overall RBC count. Red blood cell edema or the discharge of enlarged red blood cells into the circulation during stress were the causes of an elevated MCV and MCH[77]. The macrocytic nesmochromic form of anemia was suggested by the elevations in MCV and MCH as well as the decreased MCHC value[78]. Red blood cells (RBC) and mean cellular hemoglobin content (MCHC) significantly decreased following 96 h of exposure to anti-inflammatory drugs at 50 and 500 µg/L. White blood cells (WBC), hemoglobin (Hb), hematocrit (Hct), mean cellular volume (MCV), and mean cellular hemoglobin (MCH) levels all rose[79]. It was determined that rainbow trout subjected to carbamazepine (96 h LC50) exhibited considerably reduced MCV and lymphocytes than the control group[80].

The low MCV and MCH in the current study may be due to the large proportion of immature red blood cells in the bloodstream. Additionally, a rise in erythrocyte volume under stressful conditions could be another explanation[81]. White blood cells (WBCs) are crucial for innate immune defense and are involved in the regulation
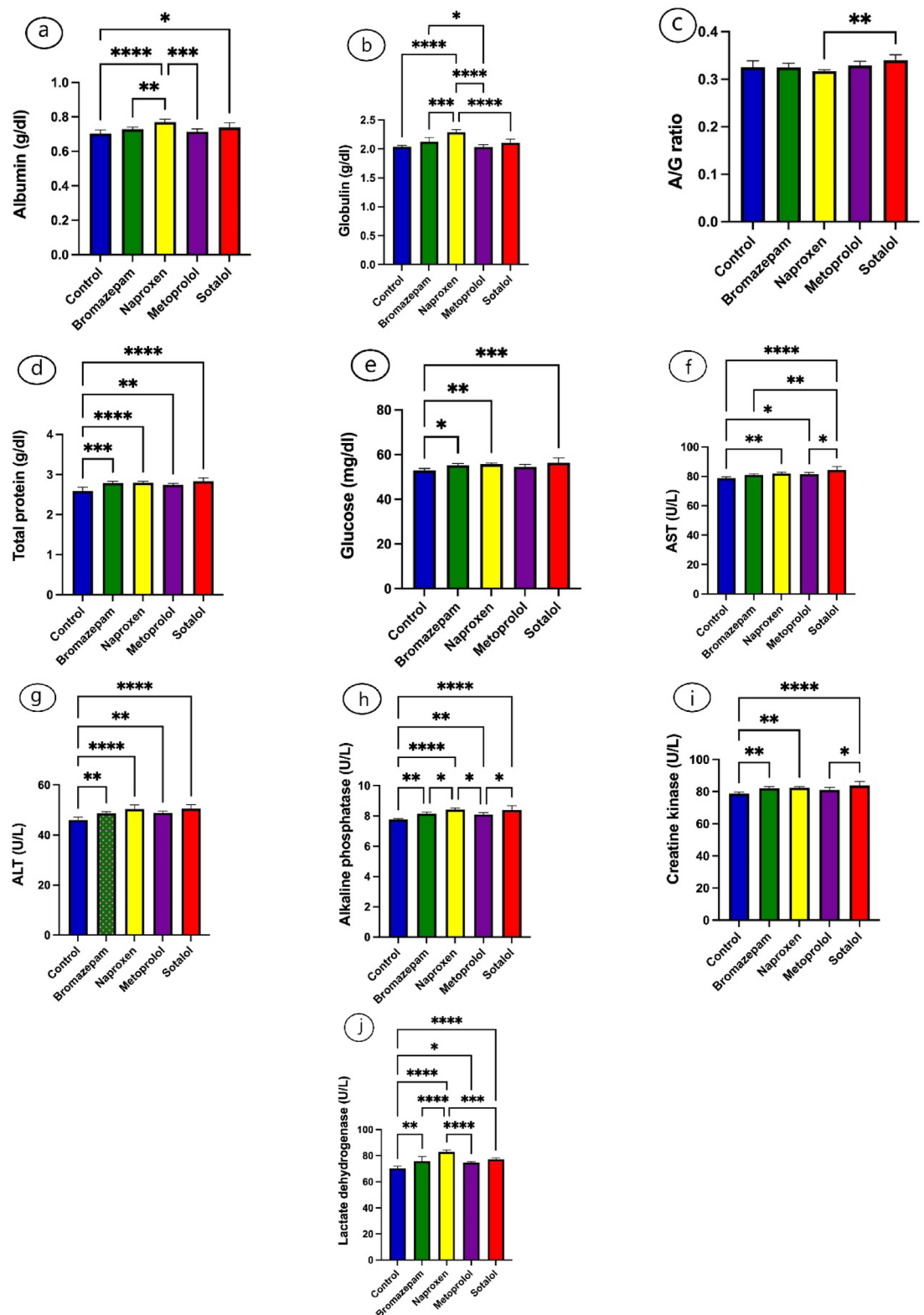
Fig. 2. The effects of bromazepam, naproxen, metoprolol, and sotalol on common carp hepatopancreas parameters. The mean ± SE is used to present the data (n = 6). Asterisks indicate significant differences from the control group (*P* < 0.05, *; *P* < 0.01, **; *P* < 0.001, ***; and *P* < 0.0001, ****).

	Control	Bromazepam	Naproxen	Metoprolol	Sotalol
CAT (Unit/mL)	16.030* ± 0.609	21.710* ± 0.565	22.380* ± 0.979	25.890* ± 0.427	25.250* ± 0.420
SOD (Unit/mL)	24.630 ± 0.962	28.140* ± 0.374	29.310* ± 0.356	34.780* ± 1.402	35.110* ± 0.590
GPX (Unit/mL)	47.850 ± 1.153	55* ± 0.806	56.840* ± 1.177	60.590* ± 1.206	60.570* ± 2.056

Table 3. Effects of pharmaceutical micropollutants on Common carp (*Cyprinus carpio*) exposure on the antioxidants biomarkers. Values are presented as mean ± standard deviation. Asterisks (*) indicate significant differences compared to the control group ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's post hoc test.
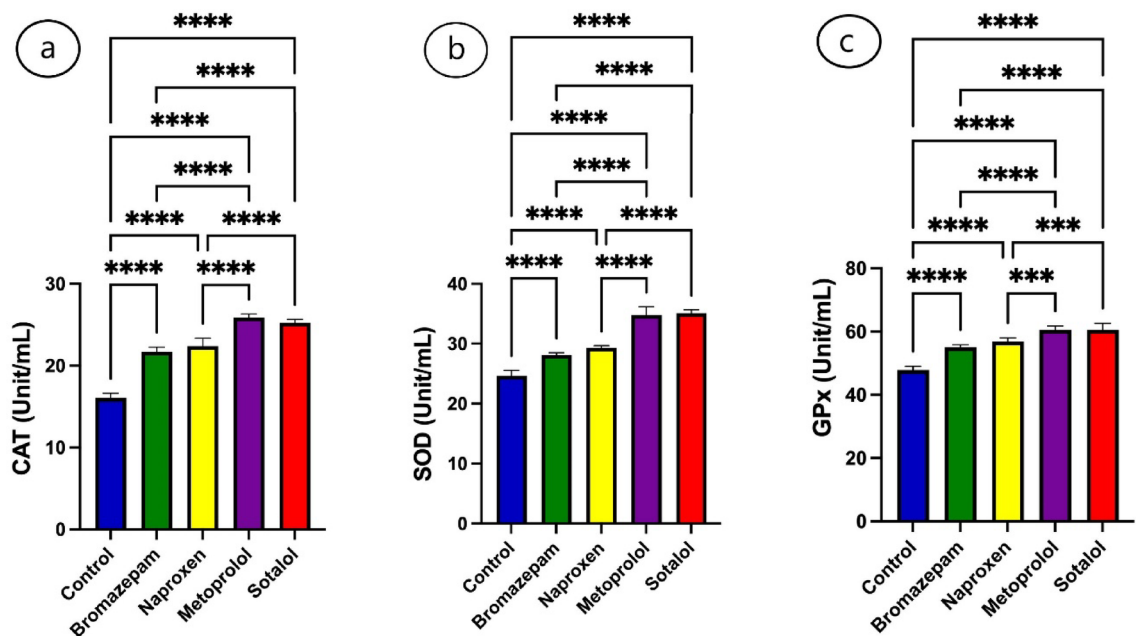

Fig. 3. The effects of bromazepam, naproxen, metoprolol, and sotalol on common carp antioxidant parameters. The mean ± SE is used to present the data (n = 6). Asterisks indicate significant differences from the control group ($P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***; and $P < 0.0001$, ****).

of immunological function in the organism; for these reasons, blood cells, especially WBCs, are frequently used as indicators of fish health (Fazio, 2019). The significant reduction in white blood cell (WBC) counts in the bromazepam and naproxen groups indicates a potential immunosuppressive effect of these compounds. WBCs are crucial for the immune response, and their reduction can make fish more susceptible to infections and diseases[82]. The significant variations in WBC counts among the tested groups underline the differential impact of these substances on the immune system. Neutrophil counts did not show significant changes between the control and treated groups; though significant differences were observed among the treated groups themselves. This suggests that neutrophils might be less sensitive to these treatments or that their responses are more variable. On the other hand, When *Claris gariepinus* was exposed to varying concentrations of atrazine and metalochlor, the results showed that both male and female *C. gariepinus* had increased levels of WBC, neutrophils, monocytes, MCH, and MCHC[83]. This is consistent with previous research on the acute hemopathological analysis of fish exposed to toxicants, specifically *Sarotherodon melanotheron*[84]. An immunological reaction to the toxicants is demonstrated by the increase in WBC, neutrophils. Studies have demonstrated that the species, chemical, and length of exposure all affect the alterations in fish haematological indices that toxicants produce and the reactions they trigger. They are also affected by the type, quality, and quantity of chemicals present in the water. They can all result in both reversible and irreversible changes to fish haematological parameters[85]. Lymphocyte counts were significantly increased in the sotalol group compared to the control, which could indicate an adaptive immune response or a compensatory mechanism to counteract the chemical effects[86,87]. Conversely, monocyte counts were decreased across all treated groups compared to controls, suggesting a general suppressive effect on this particular leukocyte population. It was reported that sulfur dioxide (SO2) was substantially linked to a decreased WBC number, especially neutrophils and monocytes. Though there was inconsistent data from in vitro research, SO2, an irritating and colorless environmental pollutant produced by numerous industrial processes, demonstrated the potential for genotoxicity and lymphocyte aberration in in vivo investigations[88,89].
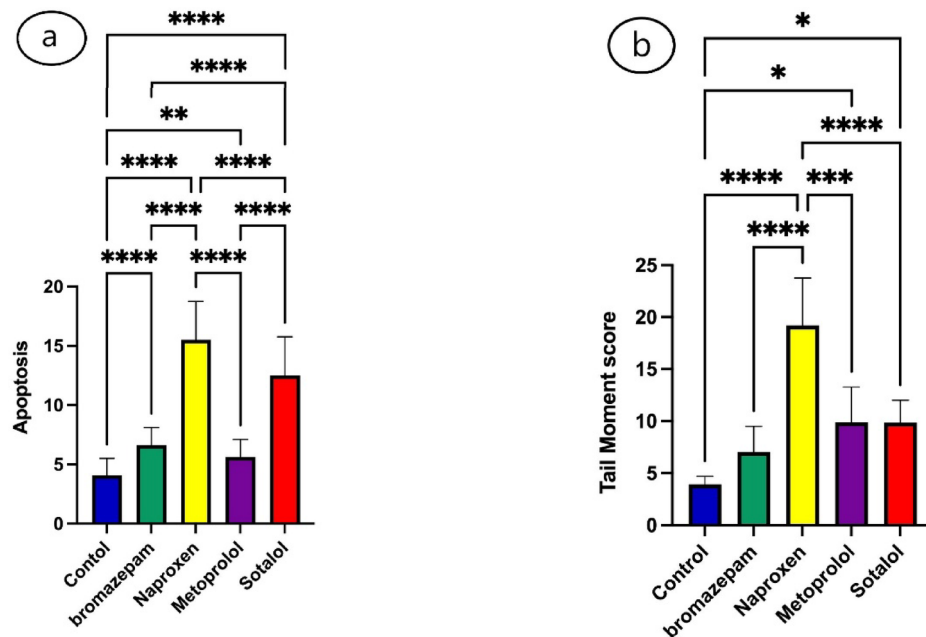
Fig. 4. (**a**) The percentage of apoptotic cells (%) in common carp exposed to bromazepam, naproxen, metoprolol, and sotalol. (**b**) The tail moment score (%) in common carp exposed to bromazepam, naproxen, metoprolol, and sotalol. The data was presented as mean ± SE, (n = 6). The significant difference between each two groups was denoted according to $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***; and $P < 0.0001$, ****.

Biochemical parameters

The hepatopancreatic function parameters presented in the study indicate significant biochemical changes in fish exposed to bromazepam, naproxen, metoprolol, and sotalol. These findings suggest potential hepatic and pancreatic toxicity associated with these pharmaceuticals, commonly found in aquatic environments due to pharmaceutical contamination. The observed increase in albumin across all test groups, with significant increases in the naproxen and sotalol groups, indicates a systemic response to these drugs. Albumin, a key plasma protein, often increases in response to liver function changes or stress[90]. While these biochemical changes are significant in carp, it is important to contextualize these findings with human toxicology. For example, naproxen is known to cause gastrointestinal and renal toxicity in humans[91], but its effects on liver enzymes (e.g., ALT, AST) are less commonly reported[92]. This discrepancy may be due to differences in metabolic pathways between humans and fish. While common carp possess cytochrome P450 enzymes (e.g., CYP1A), as evidenced by their role in detoxification and oxidative stress responses, their metabolic capacity or efficiency may differ from that of humans. For example, the activity of CYP1A in carp is significantly reduced under arsenic exposure, leading to increased oxidative stress and hepatotoxicity[93]. This suggests that, although carp have detoxification pathways, they may be less robust or more easily overwhelmed compared to humans, making them more susceptible to pharmaceutical-induced toxicity at lower concentrations[94]. These differences in metabolic resilience could explain the heightened sensitivity of carp to environmental contaminants. Pharmaceutical chemical exposure can cause stress or damage to the liver, which causes the liver to produce more albumin as a compensatory response to sustain physiological functioning and maintain osmotic equilibrium[95]. Exposure to pharmaceuticals can induce physiological stress in fish, which activates their innate immune response. This stress triggers the production of acute-phase proteins, including albumin, as part of a compensatory mechanism to address the systemic alterations caused by the stressor. Acute-phase proteins play a critical role in restoring homeostasis during such conditions, highlighting the fish's response to pharmaceutical contaminants in their environment[96,97].

Our findings indicate that pharmaceutical exposure in carp leads to an increase in albumin levels, measured at concentrations below 0.8 g/dL, during the acute-phase response. This observation aligns with the physiological alterations induced by stress, which is known to stimulate acute-phase proteins, including albumin. However, in fish like carp, the role and prevalence of albumin differ significantly compared to mammals or even other fish species, such as trout[98].

Previous studies have suggested that albumin is absent in carp plasma, with high-density lipoproteins compensating as the primary transport molecules for free fatty acids[99,100]. This evolutionary adaptation highlights the unique transport mechanisms in carp. Nevertheless, our results challenge the hypothesis of complete albumin absence, suggesting the presence of albumin or albumin-like proteins at low concentrations, which may become detectable during acute-phase responses. This increase could indicate a compensatory role for albumin-like proteins in carp under stress conditions induced by pharmaceutical exposure. Interestingly, studies on trout and other species with higher albumin levels demonstrate intermediate reliance on lipoproteins for transport, while mammals predominantly rely on albumin. These differences between fish and human albumin could be attributed to variations in plasma protein composition and binding capacities[98]. This distinction underscores

the necessity of expanding the experimental database on fish plasma proteins to refine predictive models for fish bioaccumulation and stress responses. Further studies characterizing the amino acid sequence and structure of carp albumin are warranted to elucidate its role in the observed acute-phase response.

The significant rise in globulin levels in the naproxen group suggests an immune or inflammatory response, as globulins are associated with immune function[101]. These elevations could imply an adaptive response or a compensatory mechanism due to drug-induced hepatic stress[102].

The albumin/globulin (A/G) ratio remained non-significantly changed in all groups, suggesting a balanced increase in both albumin and globulin levels. This ratio is a useful indicator of overall protein health in the blood, and its stability despite exposure to these drugs may indicate that while individual protein levels are altered, the relative balance between albumin and globulin is maintained. The glucose level increased in all tested groups compared to the control group. According to previous studies[103–105], blood glucose levels rise when fish are under stress. In *C. gariepinus* exposed to TiO2 NPs, serum glucose levels also rose as Ti concentrations and exposure times increased[106]. They supposed that the rise in glycogenolysis brought on by an increase in energy requirements as a result of metal stress may account for the rise in blood glucose.

The significant increases in total protein, alanine transaminase (ALT), alkaline phosphatase (LP), and lactate dehydrogenase (LDH) across all groups ($p \leq 0.05$) are indicative of hepatic cellular damage or increased hepatic activity[107–109]. ALT and ALP are specific markers of liver health, and their elevation typically signals hepatocellular injury or cholestasis. LDH, a marker for tissue damage, further corroborates the presence of cellular stress or damage likely induced by pharmaceuticals. The significant increases in aspartate transaminase (AST) and creatine kinase (CK) in fish exposed to bromazepam, naproxen, and sotalol ($p \leq 0.05$) further support the evidence of hepatic and possibly muscular damage[110,111]. AST is another enzyme indicative of liver damage, and its elevated levels alongside ALT strengthen the case for hepatotoxicity. Creatine kinase, primarily a muscle enzyme, suggests additional stress or damage to muscular tissues, possibly due to systemic toxicity[110,112]. According to a previous investigation, fish samples from polluted sites were shown to have significantly higher levels of ALT, AST, ALP, and LDH than fish samples from control sites without pollution[113]. This is consistent with other study that added mercuric oxide to the feeding program and led to a considerable increase in ALT and AST in the serum of *C. gariepinus* fish[114]. The collective data from these biochemical markers paint a concerning picture of the impact of pharmaceutical contaminants on aquatic life. The consistent pattern of elevated liver enzymes and proteins across different drugs suggests that these substances, even at environmental concentrations, can provoke significant physiological stress in fish. This stress could lead to compromised health, reduced growth, and potentially increased mortality, posing a threat to aquatic ecosystems. These pharmaceuticals' physiological stress may have an impact on behavior as well. It was observer a significant decrease in AST and ALT activities[75].

Antioxidant defense enzyme activity

Catalase decomposes hydrogen peroxide into water and oxygen, thus protecting cells from oxidative damage[115]. The significant rise in CAT activity implies an elevated level of hydrogen peroxide in the treated fish, reflecting the oxidative challenge posed by the exposure to these compounds[116]. SOD activity significantly increased in fish exposed to bromazepam, naproxen, metoprolol, and sotalol. SOD is a crucial enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, providing the first line of defense against reactive oxygen species (ROS)[117]. The elevated SOD activity suggests an increased production of superoxide radicals, which may result from the metabolism of these pharmaceuticals, leading to enhanced oxidative stress[118]. In addition to serving as an indicator of oxidative damage to lipids, SOD may also be responsible for the up-regulation of antioxidant enzymes[119]. Similar results were reported for common carp exposed to chemical stressor that leads to enhancing the CAT and SOD levels[116]. Juvenile rainbow trout exposed to carbamazepine showed noticeably elevated levels of SOD, CAT, GPx, and GR activity in their livers[80]. It was recorded notable changes in a number of antioxidant markers, including glutathione, lipid peroxidase, catalase, and SOD[75]. GPx reduces hydrogen peroxide and lipid peroxides to water and corresponding alcohols using glutathione as a substrate[120]. The marked increase in GPx activity further corroborates the presence of elevated oxidative stress and the requirement for enhanced detoxification of peroxides generated in response to stressor[121]. The observed oxidative stress in carp may not directly translate to humans due to differences in antioxidant defense mechanismsFor example, humans have higher baseline levels of antioxidant enzymes like SOD (superoxide dismutase) and CAT (catalase), which play a critical role in neutralizing reactive oxygen species (ROS) and mitigating oxidative stress[122,123]. This robust antioxidant defense system allows humans to better tolerate pharmaceutical-induced oxidative stress at therapeutic doses. In contrast, fish like carp may have lower baseline levels of these enzymes or less efficient antioxidant systems, making them more susceptible to oxidative damage even at lower, environmentally relevant concentrations[51,124]. These differences in antioxidant capacity highlight the need for species-specific risk assessments when evaluating the ecological impact of pharmaceuticals. This further underscores the need for species-specific risk assessments when evaluating the ecological impact of pharmaceuticals.

Apoptosis and comet assay

These results collectively highlight the differential impacts of various pharmaceuticals on common carp, with a particular emphasis on their cytotoxic and genotoxic effects. Comet, DNA fragmentation, and micronucleus tests are three different assays that can be used to assess the genotoxicity of xenobiotics on fish nucleated erythrocytes[125]. Evaluation of oxidative stress, toxicity-induced apoptosis, and other metrics of cellular damage may potentially benefit from the use of erythrocytes[126,127]. The significant increase in apoptotic erythrocytes upon exposure to the four tested compounds suggests that these substances can induce programmed cell death, potentially compromising the fish's ability to maintain normal physiological functions. The most severe

apoptosis was observed with naproxen, aligning with its pronounced genotoxic effects as indicated by the comet assay results.

While apoptosis and genotoxicity are observed in carp, these effects are not commonly reported in humans at therapeutic doses of these pharmaceuticals. Regulatory documents from the FDA and EMA do not highlight apoptosis or monocyte suppression as significant concerns for human patients[128]. While In humans, pre-approval databases for pharmaceuticals are often insufficient to detect low rates of serious hepatotoxicity, necessitating robust post-marketing monitoring systems like the FDA's MEDWATCH program[129]. Similarly, in aquatic environments, the long-term and cumulative effects of pharmaceutical contaminants on fish populations remain poorly understood due to limited monitoring and regulatory oversight. This suggests that carp may be more susceptible due to differences in cellular repair mechanisms, continuous exposure, and lower detoxification capacity. Future studies should explore these species-specific differences to better understand the ecological and human health implications of pharmaceutical contamination.

The increase in DNA damage, as measured by the tail moment, further corroborates the harmful effects of these pharmaceuticals on aquatic life[130]. The substantial genotoxicity observed with naproxen exposure is of particular concern, pointing towards its potential to cause long-term genetic damage, which could have repercussions on the health and reproductive viability of the fish population[131]. Study on Zebra fish reported that exposure to pharmaceuticals can cause both DNA and oxidative damages Neutral agarose gel electrophoresis was used to examine DNA damage photoinduced by four nonsteroidal anti-inflammatory medications[132]. Previous study demonstrates that carbamazepine exposure at environmentally relevant concentrations causes DNA damage and apoptosis in Chinese rare minnows (*Gobiocypris rarus*). The observed effects were mediated through the Ras/Raf/ERK/p53 signaling pathway. Specifically, carbamazepine exposure led to significant increases in DNA damage, apoptotic rates, and activation of key molecular pathways associated with mitochondrial apoptosis and cell cycle regulation, indicating a toxicological impact on liver tissue in both male and female minnows[133].

The results of this study regarding genotoxicity and cytotoxicity may have serious consequences for the fish's ability to reproduce. Reproductive success may be hampered by DNA damage and death in germ cells, which can result in transgenerational genetic alterations, decreased fertility, or an increase in the frequency of developmental defects in offspring[134]. Pharmaceuticals such as naproxen and bromazepam have been linked to endocrine disruption in other species, which may affect gametogenesis, hormone regulation, and spawning behavior[135]. Population losses may result from extended exposure to these pollutants in natural settings, particularly in species with low rates of reproduction or substantial parental investment.

The consequences of acute exposure were the focus of this investigation; on the other hand, it is important to consider the possible cumulative effects of long-term, low-dose exposure to pharmaceutical pollutants. Prolonged exposure to these substances may cause toxins to gradually accumulate in tissues, even at sub-lethal quantities, which could cause long-term physiological deficits[136]. Over time, such cumulative effects may lead to more pronounced implications on immunological function, development, and reproductive success in addition to exacerbating the observed hematological and biochemical changes. This raises concerns about the health of fish populations inhabiting pharmaceutical-contaminated waters, particularly as they are subject to continual exposure. The observed alterations in fish health resulting from exposure to pharmaceuticals may have wider ecological implications. Moreover, fish may be less resistant to environmental changes because of the stress brought on by pharmaceutical exposure, which might increase the effects of other stressors like habitat loss, climate change, or other contaminants.

Environmental regulations must manage the introduction of pharmaceutical pollutants into water bodies due to the possible cumulative and long-term effects on fish populations and aquatic ecosystems. To reduce the hazards to aquatic life, more stringent laws governing the disposal of pharmaceuticals and improved wastewater treatment technologies are essential[137]. Long-term, multigenerational studies should also be given priority in future research to completely understand the ecological effects of prolonged exposure to pharmacological combinations. The summary of previous studies can be shown in Table 4.

Conclusion

This study provides valuable insights into the physiological and genotoxic effects of pharmaceutical contaminants, contributing to a broader understanding of their ecological impact. By investigating diverse parameters, such as hematological and biochemical changes, the research enhances our knowledge of how these drugs affect aquatic organisms. These findings can be instrumental in improving ecological risk assessments by identifying sensitive biomarkers that signal early signs of contamination in aquatic ecosystems. To protect water quality and biodiversity, specific recommendations include the implementation of routine monitoring programs for water bodies, focusing on commonly detected pharmaceuticals and their cumulative effects. Developing and improving wastewater treatment technologies to target pharmaceutical contaminants is also essential, along with promoting policies that encourage the safe disposal of unused medications to prevent environmental release. While the implementation of strategies to reduce the entry of these pharmaceuticals into water bodies is challenging, it is feasible through a combination of technological innovations and public awareness campaigns. Advanced filtration techniques, such as reverse osmosis or activated carbon, have shown promise in removing pharmaceuticals from wastewater, but further research is needed to make these technologies more cost-effective and scalable. Collaboration between environmental agencies, industries, and policymakers will be critical in ensuring their successful adoption. Future research should focus on long-term studies to assess the chronic effects of pharmaceutical pollutants on aquatic ecosystems. These findings can further inform environmental risk assessments, guiding regulations and protective measures to safeguard aquatic life and preserve ecosystem health.

Species	Bromazepam	Sotalol	Naproxen	Metoprolol	References
Zebrafish	(0.5 mg/L) Behavioral and neuroendocrine alterations Increased inflammation				138,139
Stickleback			(0–1280 µg/L) Renal hematopoietic hyperplasia Jaw lesions Hepatocellular vacuolation		140
Labeo rohita			(1–50 µg/L) Oxidative liver damage and inflammation		141
Oryzias latipes			(0.5 mg /L) Reduced survival and endocrine disruption		142
Abramis brama and *Rutilus rutilus*			(6–32 ng/ mL) Bioaccumulation in bile		143
Zebrafish			(1–100 µg/L) Expression alterations of antioxidant genes in the intestine and liver		144
Oncorhynchus mykiss			(1.6 µg/L) Bioaccumulation in bile		26
Lumbriculus variegatus				(0.596 mg/ L) Increased lipid peroxidation concentrations	145
Oreochromis niloticus				(4×10^{-10}—4×10^{-7} M) Gill alterations and potential reproductive impairment	146
Zebrafish				(0.03–3 mg/L) Altered β-adrenergic receptor expression; oxidative stress	147
Danio rerio larvea		(0.05–500 mg L) Minimal behavioral impact			6

Table 4. Summary of some previous studies on the studied pharmaceutical micropollutants.

Data availability
Data is provided within the manuscript.

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Author contributions

Walaa M. Shaalan was involved in the conception and design of the study, data collection, analysis and interpretation of the results, drafting the manuscript, and revising it critically for important intellectual content, read and approved the final version of the manuscript and agree to be accountable for all aspects of the work. Alaa El-Din H. Sayed was involved in the conception and design of the study, data collection, analysis and interpretation of the results, drafting the manuscript, and revising it critically for important intellectual content, read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

Studies were approved by the Research Ethics Committee of the Molecular Biology Research and Studies Institute (07\2024\0056), Assiut University, Assiut, Egypt. All methods were carried out following the relevant regulations and ARRIVE guidelines.

Institutional review board statement

The ethics committee of Assuit University, Assuit, Egypt, authorized the experimental setup and fish handling in accordance with the OIE criteria for the use of animals in research.

Additional information

Correspondence and requests for materials should be addressed to A.E.-D.H.S.

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