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Original Research Article

Effects of Sub-Lethal Concentrations of Oxytetracycline and Erythromycin on Antioxidant Enzymes in *Cyprinus Carpio*

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Abstract: *Accumulation of antibiotics in freshwater bodies poses a major ecological threat, as excessive antimicrobial residues induce oxidative stress in aquatic organisms, particularly fish. The antioxidant defence system plays a crucial role in mitigating such stress responses.* ***Objective:*** *To evaluate the effects of oxytetracycline and erythromycin on the activity of antioxidant enzymes in common carp (Cyprinus carpio).* ***Methods:*** *A laboratory-based experiment was conducted using common carp collected from a local fish pond and acclimatized for 10 days under controlled conditions. Fish were exposed for 96 hours to sub-lethal concentrations of oxytetracycline and erythromycin in separate treatment groups, along with a control group. Each group consisted of three replications maintained under identical physicochemical conditions. Antioxidant enzyme activities of superoxide dismutase and peroxidase were quantified in selected organs using a visible spectrophotometer at 560 nm and 470 nm, respectively. Data were analyzed using a two-way ANOVA, followed by Tukey's HSD for mean comparisons. Correlation analysis was performed to determine associations among study variables.* ***Results:*** *A significant reduction in superoxide dismutase and peroxidase activity was observed at 72 and 96 hours of exposure in both antibiotic-treated groups compared with controls ($p<0.05$). Oxytetracycline exhibited a stronger inhibitory effect on antioxidant responses, consistent with enhanced reactive oxygen species generation. Increasing antibiotic concentration led to a marked decline in enzyme activity across both treatment groups.* ***Conclusion:*** *Acute exposure to oxytetracycline and erythromycin leads to oxidative stress in common carp by suppressing key antioxidant enzymes, indicating that antibiotic pollution in aquatic environments may compromise fish health and physiological homeostasis.*

Keywords: Oxytetracycline, Erythromycin, Antioxidant enzymes, Cyprinus carpio

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Introduction

Fish play a vital role in human nutrition and are an important source of high-quality protein in many regions of the world. Aquaculture has expanded rapidly in recent decades and now contributes substantially to global fish production, supported by advancements in water quality management, improved feeds, and better disease-control strategies (1, 2). As aquaculture continues to grow, concerns about environmental contamination have increased, particularly regarding pollutants that directly affect aquatic ecosystems. Among these pollutants, antibiotics are of major concern because they are biologically active even at low concentrations and act as environmental toxicants (3). Their persistence, bioaccumulation potential, and widespread use in aquaculture, poultry farming, human medicine, and food production have resulted in continuous discharge into freshwater bodies (4–6).

Antibiotics are frequently used in aquaculture to prevent disease outbreaks, enhance growth, and reduce economic losses (7). However, their excessive and unregulated use leads to accumulation in aquatic systems, exposing fish and other non-target organisms to harmful concentrations. The common carp (Cyprinus carpio), an ecologically and commercially important species, is particularly vulnerable to antibiotic pollution, with documented physiological and biochemical disturbances following exposure (8).

Erythromycin (ERY), a macrolide antibiotic, is commonly used against Gram-positive bacterial infections in fish. Evidence indicates that ERY exposure induces oxidative stress, genotoxicity, and cellular damage in fish tissues, including the liver, gills, and kidneys (9–11). Oxytetracycline (OTC), another widely used antibiotic in aquaculture, has also been shown to cause oxidative stress, impair physiological functions, and disrupt antioxidant systems in fish (3, 12).

Oxidative stress occurs when reactive oxygen species are generated in excess of the organism's antioxidant defense mechanisms. Enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are essential components of this defense system and serve as biomarkers for assessing the toxic effects of environmental contaminants (13,14). Excessive oxidative stress can damage cellular components, including lipids, proteins, and DNA, leading to mutations, chromosomal abnormalities, and impaired physiological function (15, 16). SOD, in particular, represents the first line of defense against superoxide radicals and plays a central role in maintaining cellular redox balance (16).

Given the increasing use of antibiotics in aquaculture and their well-established toxicological effects, understanding their impact on essential antioxidant enzymes in fish is crucial. This study, therefore, investigates the effects of sub-lethal concentrations of oxytetracycline and erythromycin on the activities of key antioxidant enzymes in Cyprinus carpio.

Methodology

This experimental study was conducted to evaluate the effects of sub-lethal concentrations of oxytetracycline and erythromycin on the activity of antioxidant enzymes in the common carp (*Cyprinus carpio*). The work was carried out in the toxicology laboratory of the Fisheries Research Farm, Faculty of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. Healthy, disease-free *C. carpio* aged 90 to 120 days were procured from the University's research farm. A total of 60 fish, with an average weight of 170 g and length of 16 cm, were acclimatized for ten days in cemented tanks under controlled environmental conditions. During acclimatization, fish were fed commercial pellet feed once daily, and water quality parameters were closely monitored. The water temperature was maintained at 26 °C throughout the day-night cycle, dissolved oxygen remained between 7 and 8 mg/L, and pH was stabilized between 7.0 and 7.5. To ensure optimal conditions, the water was renewed daily.

Following acclimatization, the fish were randomly assigned to three groups: a control group with no antibiotic exposure and two treatment groups exposed to oxytetracycline (OTC) or erythromycin (ERY). Each group included three replicates maintained under identical conditions. The selected concentrations of OTC and ERY were sub-lethal at 80 mg/L and 50 mg/L, respectively, chosen based on environmentally relevant levels and previous reports on antibiotic toxicity in aquatic organisms. Fish in the treatment groups were exposed to these antibiotics for a total duration of 96 hours. To maintain consistent exposure levels, 35% of the tank water was replaced daily, and fresh antibiotic solutions were added accordingly.

At the end of the exposure period, fish were euthanized by beheading, and three organs—liver, heart, and gills—were carefully dissected from each specimen. The tissues were immediately placed in labeled sterile polythene bags and stored at low temperature until biochemical analysis. For enzymatic assays, each organ was weighed and homogenized in phosphate buffer (pH 7.0) using a 1:4 tissue-to-buffer Ratio. The homogenates were filtered through muslin cloth to remove debris, then centrifuged at 10,000 rpm for 15 minutes at 4 °C. The resulting supernatants were collected for analysis, with 10% glycerol added to preserve enzyme activity during the procedure.

Superoxide dismutase (SOD) activity was quantified using the method of Giannopolitis and Ries (1977), which measures the enzyme's ability to inhibit the photoreduction of nitro blue tetrazolium (NBT). Peroxidase (POD) activity was determined spectrophotometrically by assessing the rate of hydrogen peroxide degradation at 470 nm following the procedure described by Zia et al. (2011). Throughout the experiment, physicochemical parameters including temperature, dissolved oxygen, pH, hardness, conductivity, calcium, magnesium, and carbon dioxide were regularly monitored using digital meters and standard analytical protocols described by the American Public Health Association (APHA, 1998).

All generated data were statistically analyzed to determine the effects of antibiotic exposure on enzyme activity. A two-way analysis of variance (ANOVA) was applied to compare mean differences among treatment groups and exposure durations. Where significant differences were detected, Tukey's HSD test was used as a post hoc comparison to identify specific group differences. Statistical analyses were performed using Statistics software version 8.1, and significance was set at $p < 0.05$.

Results

A total of 60 Cyprinus carpio were exposed to sub-lethal concentrations of oxytetracycline (OTC) and erythromycin (ERY) for 96 hours to evaluate alterations in antioxidant enzyme activity. Significant enzyme suppression was observed across all organs and exposure durations, with the magnitude of change varying by antibiotic type and tissue.

Peroxidase activity declined consistently in the liver, heart, and gills following acute OTC exposure. As shown in Table 1, liver peroxidase activity was highest in the control group (184.59 ± 0.89 U/mL) and progressively declined in Treatment 1 and Treatment 2, with the lowest value recorded in Treatment 2 (179.70 ± 1.57 U/mL). A similar pattern was observed in the heart, where POD activity decreased significantly with increasing OTC concentration ($p < 0.01$). The gills exhibited the greatest reduction in POD activity, with values ranging from 214.49 ± 0.74 U/mL in the control to 203.12 ± 3.82 U/mL in Treatment 2. These results confirm a strong dose-dependent inhibitory effect of OTC across all tissues. (Table 1).

Exposure to OTC altered SOD activity in a time- and dose-dependent manner. As presented in Table 3, heart SOD activity declined progressively with increasing exposure duration and OTC concentration. The lowest activity was observed in Treatment 2 at 96 hours (46.00 ± 1.68 U/mL), compared with 57.37 ± 0.56 U/mL at 24 hours in the control group. Similar declines were also observed in the liver and gills, indicating oxidative stress–induced suppression. (Table 3).

The gills consistently exhibited the greatest reductions in both POD and SOD activity, likely due to their direct contact with waterborne contaminants and higher metabolic activity. This organ-specific trend was consistent across Tables 1–3, confirming that the gills are the most responsive tissue to antibiotic-induced oxidative stress.

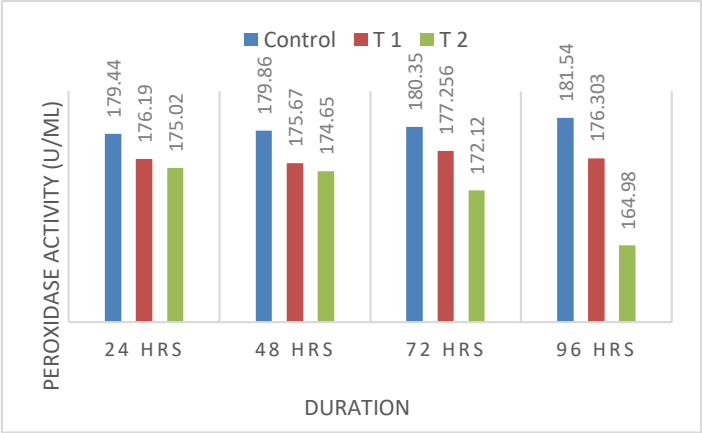

Figure 1. Peroxidase activity (U/mL) in the Heart after acute exposure to oxytetracycline (OTC) in *C. carpio*..

Table 1. Peroxidase (POD) Activity in Liver, Heart, and Gills of *Cyprinus carpio* After Oxytetracycline Exposure (Mean ± SD)

Organ	Control (U/mL)	Treatment 1 (U/mL)	Treatment 2 (U/mL)	p-value
Liver	184.59 ± 0.89	182.13 ± 1.75	179.70 ± 1.57	<0.05
Heart	180.30 ± 0.91	178.55 ± 1.43	173.77 ± 1.88	<0.01
Gills	214.49 ± 0.74	212.13 ± 1.24	203.12 ± 3.82	<0.01

Table 2. Peroxidase (POD) Activity after Erythromycin Exposure (Mean ± SD)

Organ	Control (U/mL)	Treatment 1 (U/mL)	Treatment 2 (U/mL)	p-value
Liver	170.54 ± 3.01	164.22 ± 5.21	157.37 ± 7.34	<0.05
Gills	209.88 ± 0.97	208.78 ± 1.06	203.84 ± 4.26	<0.01

Table 3. Superoxide Dismutase (SOD) Activity in Heart after Oxytetracycline Exposure (Mean ± SD)

Duration	Control (U/mL)	Treatment 1 (U/mL)	Treatment 2 (U/mL)
24 h	57.37 ± 0.56	55.23 ± 1.13	54.52 ± 1.17
48 h	57.19 ± 1.01	54.09 ± 0.29	50.99 ± 1.78
72 h	51.14 ± 0.41	49.65 ± 0.52	47.95 ± 1.76
96 h	51.62 ± 0.84	51.00 ± 0.82	46.00 ± 1.68
Mean ± SD	54.33 ± 3.41	52.49 ± 2.61	49.87 ± 3.72

Across both antibiotics, the observed pattern of tissue sensitivity was: Gills >

Discussion

The results of the study evaluating the alteration in antioxidant enzyme activity in *Cyprinus carpio* exposed to sub-lethal concentrations of oxytetracycline (OTC) and erythromycin (ERY) provide significant insights into the impact of antibiotics on oxidative stress responses in aquatic organisms. Analysis of peroxidase (POD) and superoxide dismutase (SOD) enzyme activities across various tissues provides deeper insight into antibiotic-induced oxidative stress, shedding light on the intrinsic regulation of these vital biochemical processes in fish.

As shown in Table 1, peroxidase activity in the liver, heart, and gills declined with increasing OTC concentrations, demonstrating a strong dose-dependent suppression ($p < 0.05$) across the studied tissues. This decline aligns with findings by Kondera et al. (17), who reported that OTC treatment led to significant disturbances in the antioxidant systems of *Cyprinus carpio*. The greatest reduction in POD activity was observed in the gills, which are particularly vulnerable to oxidative stress due to their direct contact with aquatic pollutants, as reported by other studies (18).

Compared with Table 2, ERY showed a milder but still significant reduction in POD activity, particularly in the liver and gills ($p < 0.01$). This differential response to OTC and ERY may be explained by the specific mechanisms of action of each antibiotic; ERY appears to engage the antioxidant machinery less aggressively than OTC. While there are few comparative studies directly corroborating this, it is generally accepted that OTC leads to greater antioxidant depletion in the cases examined (17).

The concentrations of SOD also decreased in a time- and dose-dependent manner, particularly in heart tissue (Table 3). The reduction of SOD activity at 96 hours post-exposure (46.00 ± 1.68 U/mL in Treatment 2) highlights acute oxidative stress resulting from prolonged exposure to OTC, which supports findings from Sherif et al. (19), indicating that antibiotic stress correlates with enhanced oxidative damage in fish tissues. Furthermore, while Sarkar et al. Sarkar et al. (20) reported an increase in SOD activity under different experimental conditions; the context of antibiotic exposure leading to oxidative stress remains significant in the current findings.

The trends noted in the current data reiterate the organ-specific sensitivity to antibiotic exposure, with the gills consistently showing the most pronounced activity suppression in both POD and SOD, likely due to their adaptation to aquatic environments and higher metabolic rates (18, 21). This observation reinforces the idea that gills are particularly susceptible to the nuances of waterborne contaminants.

In summary, the findings reported in Tables 1 through 3 are consistent with the recent literature, which affirms the protective role of antioxidant enzyme systems in fish and their vulnerability to oxidative stress induced by pharmaceutical contaminants such as OTC and ERY. This comprehensive understanding underscores the need to evaluate the ecological consequences of antibiotic use, especially in aquaculture settings. It highlights the need for more extensive research on mitigating oxidative stress in aquatic organisms. These findings will be instrumental in advancing our knowledge regarding fish health and environmental sustainability.

Conclusion

This study demonstrates that acute exposure to sub-lethal concentrations of Oxytetracycline and Erythromycin significantly disrupts the antioxidant defense system of fish. Oxytetracycline produced a stronger inhibitory effect than Erythromycin, with superoxide dismutase showing greater sensitivity than peroxidase across all examined organs. Increasing antibiotic concentrations led to a progressive decline in SOD activity, with the organ-specific response following the pattern liver < heart < gills. These findings highlight the vulnerability of aquatic organisms to antibiotic contamination and underscore the need for stricter control of antibiotic discharge into freshwater ecosystems.

Declarations

Data Availability statement
All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate
Approved by the department concerned. (IRBEC)

Consent for publication
Approved

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Conflict of interest

The authors declared no conflict of interest.

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All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

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