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Assessment of Erythromycin-induced behavioural responses in *Cyprinus carpio*

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Abstract
The extensive utilization of antibiotics and other pharmaceuticals has led to a concerning transformation of polluted aquatic ecosystems globally. Among these antibiotics, there is an increasing detection of erythromycin, a representative of macrolides, which induces toxic effects on non-target aquatic organisms. The ecotoxicity of erythromycin in freshwater was assessed using *Cyprinus carpio* during a 96-hour acute exposure. Toxicity was evaluated using a sublethal concentration of 2.5 mg/L of erythromycin, with a focus on behavioural endpoints, as behavioural biomarkers are non-invasive, ecologically relevant, detectable at low toxic concentrations, and provide early warning signals regarding the health of the exposed population. Behavioural responses were monitored at 24, 48, 72, and 96 hours. The observed behavioural alterations included hyperactivity, increased operculum movement and swimming rate, dark pigmentation, excessive mucus secretions, loss of equilibrium, surface air gulping, aggregation, lethargy, caudal bending, responsiveness to stimuli or sensitivity, and shedding of scales. These behavioural responses may indicate respiratory impairment, neurotoxic effects, and defensive responses to minimize the irritation caused by erythromycin. Consequently, the findings suggest that behavioural biomarkers serve as a crucial tool for observing and evaluating environmental health conditions. There is an urgent need to monitor erythromycin concentration and its toxicity in aquatic ecosystems.

Keywords: Pharmaceuticals, antibiotics, erythromycin, macrolides, ecotoxicity, behavioural biomarkers, *Cyprinus carpio*

Introduction

Water and aquatic ecosystems are fundamental to life and form a major component of the global environment, encompassing freshwater and marine systems. However, increasing anthropogenic activities have intensified aquatic pollution, posing significant risks to water quality, aquatic organisms, and human health (Zandaryaa *et al*., 2025) [31]. With increasing industrialization there is an alarming emergence of emerging pollutants which has ability to persist, bioaccumulate, and effect the ecology of aquatic ecosystem and humans (Zareitalabad *et al*., 2013; Liu and Wong, 2013).

Pharmaceuticals are also referred to as emerging pollutants, micropollutants, or chemicals of emerging concern (Liu *et al*., 2019) [14]. These compounds possess complex molecular structures, diverse functionalities, and specific physicochemical and biological properties (Kümmerer *et al*., 2004). In a world market of pharmaceuticals India has emerged as one of the top five pharmaceuticals market. Recent studies have reported the presence of pharmaceuticals in major Indian river systems, including the Ganges, Brahmaputra, and Arkavathi rivers (Sharma *et al*., 2019; Kumar *et al*., 2019; Gopal *et al*., 2020) [5, 23]. The ecological impacts of pharmaceuticals are multifaceted and can influence various physiological and biochemical processes in aquatic organisms (Porretti *et al*., 2022) [17]. Among active pharmaceutical ingredients (APIs), antibiotics are of particular concern due to their ability to alter microbial community structure and promote the development of antimicrobial resistance, posing significant environmental and public health risks (Boxall *et al*., 2012; Singer *et al*., 2014) [2, 26]. Antibiotics are natural or synthetic compounds known for their antimicrobial activity (Peng *et al*., 2023) [16] and are widely used for the treatment and prevention of bacterial infections. In addition to therapeutic applications, antibiotics are extensively used in aquaculture and livestock production to prevent disease outbreaks and enhance growth performance in cultured species (Gothwal *et al*., 2015; Sarmah *et al*., 2006) [6, 21]. Due to excessive usage and discharge of untreated wastewater, antibiotic residues are widely dispersed in aquatic and terrestrial ecosystems (Santos *et al*., 2013; Sui *et al*., 2015) [20, 27]. Through biomagnification, these compounds can accumulate in the food chain, posing serious risks to wildlife and human health (Jin *et al*., 2024) [7]. Among the antibiotics currently used for human treatment worldwide, macrolide antibiotics—such as erythromycin, clarithromycin, and azithromycin—are frequently misused and have been included as high-risk substances in the European Union Monitoring Watch List under Regulation 2018/840/EU (Baranauskaite-Fedorova *et al*., 2023) [1]. The World Health Organization ranks macrolides among the most critically important antibiotics for human medicine (Fedorova *et al*., 2023).

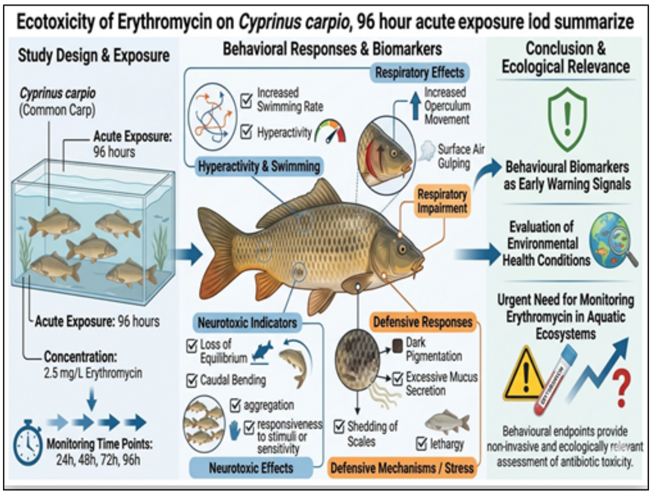
Erythromycin (ERY), a representative macrolide antibiotic, is a broad-spectrum antimicrobial agent derived from *Saccharopolyspora erythraea* and remains an important therapeutic compound with extensive antibacterial activity (Platon *et al*., 2025) [15]. Erythromycin was originally discovered by McGuire *et al*. in 1952. It is widely used for treating infections of the respiratory tract, skin, and soft tissues and has also been explored for applications in bone cements and emerging therapeutic fields such as myotonic dystrophy, cancer, and COVID-19 treatment (Hamoya *et al*., 2017; Nakamori *et al*., 2024; Platon *et al*., 2022; Wang *et al*., 2022; Yongsheng *et al*., 2011). The presence of erythromycin in aquatic ecosystems contributes to the development of antimicrobial resistance and ecotoxicological effects (Lupoi *et al*., 2025) [15]. ERY has been identified as an antibiotic of particular environmental concern due to its high consumption, continuous discharge, persistence, and toxic properties (Zuccato *et al*. 2000; Zuccato *et al*. 2006; Johnson *et al*. 2015; Liu *et al*. 2018) [8, 13, 32, 33]. ERY was detected so far in different aquatic matrices in levels ranging from the ng/L to the µg/L. experimental studies have demonstrated that erythromycin exposure can induce oxidative stress and genotoxic effects in fish. For example, behavioural changes, cytotoxicity, genotoxicity, aqueous exposure to erythromycin increased thiobarbituric acid reactive substances (TBARS) in liver tissues, reduced antioxidant capacity, caused DNA damage, and increased erythrocytic nuclear abnormalities in juvenile rainbow trout (*Oncorhynchus mykiss*) (Rodrigues *et al*., 2016) [18].

Behaviour is an organism-level effect defined as the action, reaction, or functioning of a system under a set of specific circumstances. Behaviour is the result of the interaction between internal and external stimuli (Kane *et al*., 2004) [9]. Bioassay based on behaviour is faster, more sensitive and ecologically more relevant because behavioural biomarkers are a non-invasive method for evaluation of adverse effects of stressor parameters and do not require killing fish or the use of expensive tools. Change in behaviour can be noticed when the animal is exposed to a chemical concentration below than that can cause mortality and the behavioural changes shown by any organism in response to any chemical, depend upon their mode of action (Sharma *et al*., 2015) [24]. Any change in fish behaviour give information and knowledge regarding behavioural alterations which can be related to physiological biomarkers in aquatic species (Sharma *et al*., 2019) [25].

Among freshwater fish species, *Cyprinus carpio* (common carp) was selected as the experimental model in the present study due to its sensitivity to environmental contaminants, adaptability, ecological relevance and ease of maintenance under laboratory conditions. Fish occupy higher trophic levels in aquatic ecosystems and provide an integrated representation of environmental health, making them effective bioindicators of ecosystem status (Yancheva *et al*., 2026) [30].

The present study is designed to fulfill the research gap on the erythromycin induced behavioural alterations in freshwater fish (*C. carpio*). By monitoring the aspect of behavioural changes, the present study raises awareness and concern regarding the escalating toxicity of erythromycin or antibiotics in aquatic life. It shows an urgent need for the deeper studies risk assessment of erythromycin or its residues in future to improve the quality of water and health risk of human.

Graphical Abstract:



Material and methods

Fish collection and Acclimatization

Healthy and active specimens of fish *Cyprinus carpio* of average length and weight were procured from the fish farm of department of fisheries, DGCN COVAS, CSKHPKV, and Palampur H.P. and safely transported to the laboratory. Fish were treated with 0.02% of $KMnO_4$ for 2 minutes to remove dermal infection. Fish were acclimatized for 15 days in glass aquaria having dechlorinated water under laboratory conditions. Fish were fed with commercial feed @ 2% of body weight twice daily and feeding was stopped 24hrs prior to experiment to prevent the interference from metabolic waste.

Chemical

Erythromycin (CAS: 643-22-1; purity: 98 %) a macrolide antibiotic, was purchased from a local store. The sub-lethal concentration of erythromycin was selected based on the reported LC50 value for *C. carpio* was fixed at 2.5 mg L-1 of water for exposure (Rodrigue *et al*., 2018). A stock solution of erythromycin was prepared using distilled water, and the required working concentrations were obtained through appropriate dilution during the experimental period.

Experimental design

A total of 75fish of desired morphometric characteristics (Table 1) were randomly allocated into five experimental groups, comprising one control group and four treatment groups corresponding to different exposure durations. Each group consisted of 15 fish maintained in triplicate, with five fish per replicate. The experiment was carried out in glass aquaria of 60L capacity which were cleaned with 1% potassium permanganate (KMnO4) and dried under sunlight. Each aquarium is filled with 50L of dechlorinated water.

Table 1: Morphometric characteristics of experimental fish

Fish species	Average length (in cm)	Average weight (in g)
Common carp (*Cyprinus carpio*)	18.4 ± 0.5 cm	77.5 ± 7.1 g

Behavioural biomarkers

Behavioural alterations are the changes in behavioural response of fish to the changing environment or deteriorating water quality. Hyperactivity, increased operculum movement and swimming rate, dark pigmentation, excessive mucus secretions, loss of equilibrium, surface air gulping, aggregation, lethargy, caudal bending, responsiveness to stimuli or sensitivity, and shedding of scales were selected as the behavioural biomarkers for the present study.

Results

The exposure of the experimental group to sublethal concentration of erythromycin resulted in significant time-dependent behavioural and morphological changes from 24h to 96h compared to the control group. Control or untreated fish exhibited normal behaviours like a normal rate of swimming with no signs of stress, mild opercular movement, responding to low stimulus during the experiment. While a number of behavioural alterations have been observed in the treated groups from 24h to 96h are shown in Table 2.

When *Cyprinus carpio* fish were put in water with 2.5mg/l of erythromycin for 96 hours, their behaviour changed significantly compared to the healthy fish in the control group. Right from the start, during the first 24 to 48 hours, the fish became very hyperactive, swam faster, and kept gulping for air at the surface, showing they were immediately stressed. They also turned darker in colour and started losing some scales early on. By 48 hours, mucus secretion became more pronounced, likely as a protective response to the antibiotic in the water.

By the 72-hours, the stress seemed to reach its peak point and a significant instability was observed. This was the only time the fish actually lost their balance and showed "caudal bending," where their tails looked curved or stiff. They also showed a fluctuated behaviour of being very sluggish (lethargy) and being high responsiveness to stimuli or overly sensitive to any movement in the water. They often exhibit corner behaviour.

By the final 96-hour mark, some of the frantic swimming slowed down, but their physical bodies showed the most damage. They were producing a lot of protective mucus and losing a large number of scales, which suggests the antibiotic was irritating their skin. Throughout the whole four days, the treated fish breathed much faster and stayed huddled (aggregated) together more than the normal fish which is a common defensive behaviour in stressed aquatic species, proving that the erythromycin caused them continuous discomfort and a constant "fight-or-flight" response.

Table 2: Different behavioural responses observed in *C. carpio* during 96h exposure of 2.5mg/l erythromycin.

Sr. No	Parameters	ctrl	24h	48h	72h	96h
1.	Hyperactivity	-	+++	++	++	+++
2.	Rate of swimming	+	++	+	+++	+++
3.	Surface gulping	-	++	+	++	+
4.	Corner behaviour	-	-	+	+	-
5.	Operculum movement	+	++	++	++	++
6.	Loss of balance	-	-	-	+	-
7.	Caudal bending	-	-	-	+	-
8.	Lethargy	-	-	+	+	-
9.	Responsiveness to stimuli	-	++	+	++	+++
10.	Pigmentation	-	+++	+++	++	+
11.	Mucus secretion	+	+	+++	++	+++
12.	Scale shedding	-	+	++	++	+++
13.	Aggregation	-	+	+	+	+

(-) none, (+) mild, (++) moderate, (+++) strong

Discussion

ERY was included in the list of class I—high-priority pharmaceuticals, requiring future monitoring and the development of specific ecotoxicological studies to address its toxic effects (Voogt *et al*., 2009) [29]. One of the earliest and most noticeable indicators of ecotoxicity induced by ERY is alteration in fish behaviour. As they provide integrative measures of neurotoxicity, behavioural changes are referred as early toxicity indicators (Scott and Sloman, 2004) [22]. Behavioural alterations are the result of adaptation to changing environment. Effects of pharmaceutical on behaviour are of direct ecological importance, as behaviours are tightly linked to individual fit ness and population persistence (Brodin *et al*., 2014) [3]. The swimming behaviour of fish is closely related to all the life activities of fish. Hyperactivity in fish exposed to antibiotics is often linked to the inhibition of acetylcholinesterase (AChE). When this enzyme is blocked, acetylcholine builds up in the nervous system, causing overstimulation and erratic movement (Sharma *et al*., 2019) [25]. Although behavioural changes such as anorexia have been observed in rainbow trout (*Salmo gairdneri Richardson*) induced by erythromycin (Hicks and Geraci, 1984), erythromycin treatment has been shown to impair locomotion in medaka and zebrafish, associated with neuroendocrine disorders and impaired vision. Caudal bending can also result from severe muscle tetany or mineral imbalances caused by the drug's interference with ion regulation. (Li and Zhang, 2020) [12]. Increased surface gulping and rapid gill (operculum) movement indicate that the fish are struggling to get enough oxygen. Erythromycin can cause structural damage to gill lamellae, reducing the efficiency of gas exchange. By moving to the surface or gasping, the fish are seeking the "surface film" where dissolved oxygen levels are typically highest, a common response to chemical-induced respiratory stress (Rodrigues *et al*., 2019) [19]. Excess of mucus secretion and shedding of scales are acting as protective barriers against the erythromycin. The peak in mucus secretion at 96 hours is a defensive mechanism. Fish produce extra mucus to coat their bodies and gills, attempting to dilute the toxicant and prevent it from entering the bloodstream. Constant scale shedding and changes in pigmentation indicate that erythromycin is physically irritating the skin. Prolonged mucus production eventually fails to protect the fish, leading to the sloughing off of the epidermis and scales (Dash *et al*., 2018) [4]. The loss of balance and tail bending observed at 72 hours are severe signs of neurotoxicity. This suggests the antibiotic has crossed the blood-brain barrier or affected the vestibular system, which controls posture and swimming coordination (Tong *et al*., 2025) [28].

Conclusion

This study shows that exposing *Cyprinus carpio* to 2.5 mg/l of erythromycin causes stress response over 96 hours. The fish didn't just change their behaviour; they showed a progressive decline in health. It started with immediate panic—hyperactivity and gasping for air—and moved into more serious neurological issues like losing their balance by the third day. By the end of the four days, the physical damage was obvious, with the fish losing scales and producing heavy layers of mucus to protect themselves. These results prove that even at this dosage, the antibiotic acts as a significant environmental stressor that disrupts the normal life, movement, and physical well-being of the fish.

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