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RESEARCH ARTICLE

Fluoxetine-Induced Behavioral Impairment and Hepatic Biotransformation Changes in the Freshwater Carp Labeo Rohita

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Abstract: Fluoxetine (FLX), a widely prescribed antidepressant for depression, obsessive-compulsive disorder (OCD), and panic disorders, is frequently detected in aquatic ecosystems, where it can interfere with neuronal signaling and vital physiological processes in non-target species. However, limited information is available on its chronic toxicity in fish. In this study, the freshwater carp Labeo rohita was exposed to environmentally relevant concentrations of FLX (1, 10, and 100 µg/L) for 35 days, with sampling on the 7th, 14th, 21st, 28th, and 35th days. Behavioral endpoints, gill DNA damage, and hepatic ethoxyresorufin O-deethylase (EROD) activity were investigated as biomarkers of exposure and effect. FLX exposure caused significant behavioral impairments, including a reduction of up to 88% in locomotor activity, as well as decreased feeding and reduced numbers of active individuals. Gill DNA damage increased in a concentration- and time-dependent manner, indicating genotoxic stress. In addition, hepatic EROD activity was significantly altered, suggesting modulation of detoxification pathways by FLX. Collectively, these findings highlight that chronic FLX exposure can induce behavioral disruption, genotoxicity, and hepatic biotransformation changes in L. rohita, underscoring its ecotoxicological risk to aquatic organisms.

Keywords: Ecotoxicology, Labeo rohita, Biomarkers, FLX, Behavior, DNA damage, EROD activity.

INTRODUCTION

Fluoxetine (FLX) is one of the most widely prescribed antidepressants, functioning as a selective serotonin reuptake inhibitor (SSRI) that maintains serotonin concentrations in the synaptic cleft (Winder *et al.*, 2012). It is recommended for the treatment of several psychiatric disorders, including major depressive disorder, obsessive-compulsive disorder, bulimia nervosa, panic disorder, and premenstrual dysphoric disorder. (Gao *et al*., 2025). Due to its extensive global use and limited removal in wastewater treatment plants, FLX residues are now recognized as contaminants of emerging concern (Richendrfer & Creton, 2017). Detected surface water concentrations vary widely, ranging from 0.03 ng/L in the Guadalquivir River, Spain (López-Serna *et al*., 2013), to 300 ng/L in Crabtree Creek, North Carolina, USA (McEachran *et al*., 2018).

Pharmaceuticals, particularly neuroactive drugs, are of ecotoxicological concern because conventional sewage treatment processes are not designed to fully remove them (Aus der Beek *et al*., 2016). Their continuous input leads to chronic exposure in aquatic organisms. Earlier studies have shown that FLX exposure can alter fish behaviors critical to survival, including swimming activity, feeding, and predator evasion (Dorelle *et al*., 2020; Hossain *et al*., 2020). Such changes can compromise ecological fitness by reducing energy acquisition and increasing vulnerability to predation. In parallel, FLX has been linked with genotoxic responses, such as increased DNA strand breaks, indicating oxidative and neurotoxic stress in exposed organisms (Duarte *et al.*, 2020). At the biochemical level, cytochrome P450-dependent enzymes like ethoxyresorufin O-deethylase (EROD) are sensitive biomarkers of pharmaceutical exposure, reflecting alterations in hepatic detoxification and xenobiotic metabolism.

Labeo rohita,(*L.rohita*) a freshwater carp of high economic and ecological significance in South Asia, is an established model in toxicological research. Its sensitivity to environmental pollutants and wide use in aquaculture make it suitable for evaluating long-term contaminant effects.(Ullah. *et.al*., 2025).However, data on the chronic impacts of environmentally relevant concentrations of FLX on *L. rohita* remain scarce, especially in relation to behavioral alterations, genotoxic damage, and detoxification responses.

This study aimed to address these knowledge gaps by assessing the chronic sub-lethal effects of FLX on *L. rohita*. Behavioral endpoints such as locomotor activity and feeding were monitored to evaluate neurobehavioral disruptions. DNA damage in gill tissues was analyzed as an indicator of genotoxic stress. Additionally, hepatic EROD activity was measured to determine potential modulation of detoxification pathways. Together, these biomarkers provide a multi-level perspective on the ecological risks of FLX to freshwater fish.

MATERIALS AND METHODS

Test chemical

- FLX(CAS: 114–07-8, potency: ≥ 850 µg per mg) was purchased from Sigma -
- Aldrich Corporation (USA), and other chemicals used in the study were purchased from Hi-
- media Laboratory Ltd., Mumbai, India. The stock solution was prepared by dissolving 1g of FLX in 1000 mL of dechlorinated tap water.

Fish sampling and acclimation

The fingerlings of *Labeo rohita*, a large carp species native to India, were procured from the Tamil Nadu Fisheries Development Corporation Limited, Tamil Nadu, India. The fish had an average length of 6–7 cm and an average weight of 4–5 g. Before experimentation, they were acclimatized to laboratory conditions for two weeks. During acclimatization, the physicochemical parameters of the water were carefully monitored and maintained within optimal ranges: temperature at 27.4 ± 1.2 °C, pH at 7.0 ± 0.06, dissolved oxygen at 6.6 ± 0.4 mg/L, total alkalinity at 18.2 ± 8.2 mg/L, total hardness at 18.6 ± 1.3 mg/L, and salinity at 0.4 ± 0.02 ppt. The fish were fed once daily with a diet consisting of rice bran and groundnut oil cake provided ad libitum. To maintain water quality, the tank water was renewed every 24 hours, with simultaneous removal of residual feed and fecal matter.

The experiment included FLX (FLX) concentrations and their metabolites within the range of environmentally relevant exposures. FLX stock solutions were prepared using milliQ-grade water and stored at 20 °C. Daily water renewal was performed to maintain consistent FLX concentrations in the tanks, with appropriate adjustments applied as needed. Fish mortalities were recorded daily, and water quality parameters such as temperature, salinity, pH, and ammonia levels were monitored regularly. Feeding was withheld 24 hours prior to the start of the exposure period, and no mortality-related clinical symptoms were observed during the maintenance phase.

Chronic Toxicity Study

Fingerlings of *Labeo rohita* were exposed to different concentrations of FLX (FLX) over a 35-day period. The experimental groups included a control group (no exposure) and FLX-treated groups at 1, 10, and 100 µg/L. Exposures were conducted at 7-day intervals. Each group consisted of 50 fingerlings maintained in 100 L of water, with three replicates per treatment. FLX was administered daily, and standard husbandry protocols, including feeding, removal of excess feed and waste, and daily water renewal, were followed. To minimize stress, fish were transferred to individual behavioral observation tanks on the 35th day of exposure and allowed to acclimate for approximately 10 minutes prior to observations.

Behavioral Observation of *L. rohita*

To reduce stress from visual contact or human presence, tanks were fully covered during experimental trials. During feeding tests, individual tanks received 10 fish each and were recorded for 5 minutes. Behavioral parameters recorded included the number of actively feeding fish, total duration of movement, and latency to initiate the first movement. Gill, liver, and brain tissues were collected from control and FLX-exposed groups at 7, 14, 21, 28, and 35 days. These samples were immediately processed for toxicity assessment, with gills used to evaluate DNA damage and liver tissues for protein determination and Ethoxyresorufin-O-deethylase (EROD) activity

Ethoxyresorufin-O-deethylase (EROD) activity in Liver

In determining ethoxy resorufin-O-demethylase (EROD) activity in liver tissue Fernandes *et al.* (2002) method was used with minor modifications. 190 mL of 7-ethoxy resorufin solution (0.1 mg mL-1 in 100 mM phosphate buffer, pH 7.4) and 100 mL of sample were added to start the reaction, which was then allowed to run at 30 °C for 20 minutes. Using resorufin sodium salt as a reference, the fluorescence from 7-hydroxy resorufin was measured at 530 and 585 nm excitation and emission wavelengths. The amount of resorufin(pmol) produced per mg of total protein per minute of reaction time was used to calculate activity.

DNA damage analysis in gills

DNA strand breaks in gill tissue were quantified following the alkaline precipitation method of Olive (1988) with modifications. Briefly, 50 µL of gill homogenate was mixed with 250 µL of 2% SDS solution containing 10 mM EDTA, 10 mM Tris (pH 12.4), and 50 mM NaOH, followed by incubation at 60 °C for 10 min. After addition of 250 µL KCl (0.12 M) and cooling on ice, the mixture was centrifuged at 8000 g for 5 min at 4 °C. DNA content in the supernatant was quantified fluorometrically at excitation/emission wavelengths of 360/460 nm after staining with Hoechst dye.

Statistical analysis

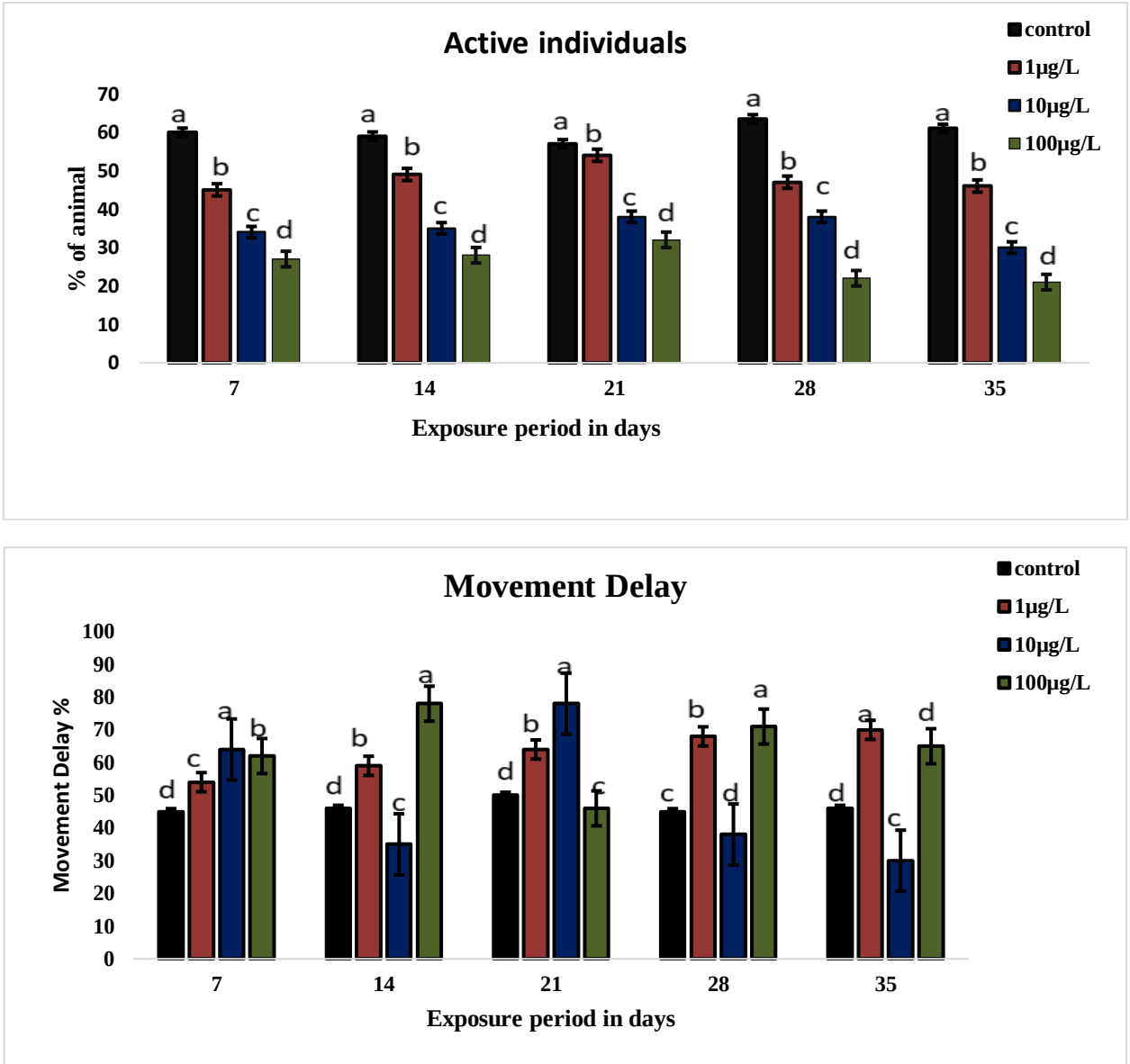
All data are shown as mean ± standard error (S.E.). Differences between control and treatment groups (Treatment I, II, and III) were tested using Duncan's Multiple Range Test ($P < 0.05$). Normality and variance homogeneity were checked using the Shapiro–Wilk and Levene tests, and data were transformed if needed. Biomarker responses in replicate tanks ($n = 3$ per treatment) during the 35-day experiment were analyzed using one-way ANOVA. DNAd results from all tanks were included because replicate responses were similar. Post hoc Tukey tests were used to compare treatments. In the 7-day experiment, sample sizes were $n = 3$ for EROD and $n = 12$ for DNAd and behavior. Behavioral data were analyzed with Fisher's exact test (active and feeding fish) and Kruskal–Wallis test (active time and movement delay), followed by post hoc comparisons. All

analyses were done in R (RStudio Team, 2016) with significance set at 0.05.

RESULT

Water quality parameters remained consistent across all tanks and throughout the exposure period. Daily measurements recorded temperature (20.7 ± 0.2 °C), salinity (18.1 ± 0.1), pH (7.3 ± 0.2), and conductivity (26.8 ± 0.2 µS), with ammonia levels maintained below 0.2 mg/L. During the 35-day experiment, three fish died, one each from the control, 10 µg/L, and 100 µg/L FLX treatment groups indicating that mortality remained below 10%, in accordance with OECD guidelines.

Chronic FLX exposure caused significant behavioral alterations in *L. rohita*. The percentage of active individuals decreased in a clear concentration-dependent manner across all sampling days (Fig. 1). The control group consistently maintained the highest activity levels (59–63%), while fish exposed to 1 µg/L showed moderate reductions (49–54%), 10 µg/L showed further decline (40–47%), and 100 µg/L showed the most pronounced suppression (30–39%). Complementary to this, latency to initiate movement increased with rising FLX concentrations **(Fig. 1,2).** Control fish responded rapidly with minimal delay, whereas 1µg/L exposure caused a moderate increase, and 10 µg/L and 100 µg/L groups showed markedly prolonged delays, with the highest concentration producing the longest latency. These behavioral disruptions were statistically significant ($p < 0.05$) and consistent over the 35-day exposure period.



Figs.1 and 2. Behavioral responses of *Labeo rohita* following sub-chronic exposure to fluoxetine (FLX) for 35 days. Bars represent mean values, and error bars indicate the standard error (SE) of five replicates. Significant differences are shown at $P < 0.05$.

Hepatic ethoxyresorufin-O-deethylase (EROD) activity showed a non-monotonic response to FLX exposure (**Fig. 3).** At 1 µg/L, EROD activity was significantly induced compared with control, reflecting activation of phase I detoxification enzymes. At 10 µg/L, activity remained slightly elevated but lower than the induction observed at 1 µg/L. In contrast, 100 µg/L exposure resulted in significant inhibition of EROD activity relative to both control and lower concentrations, suggesting suppression of detoxification pathways at higher exposure levels.

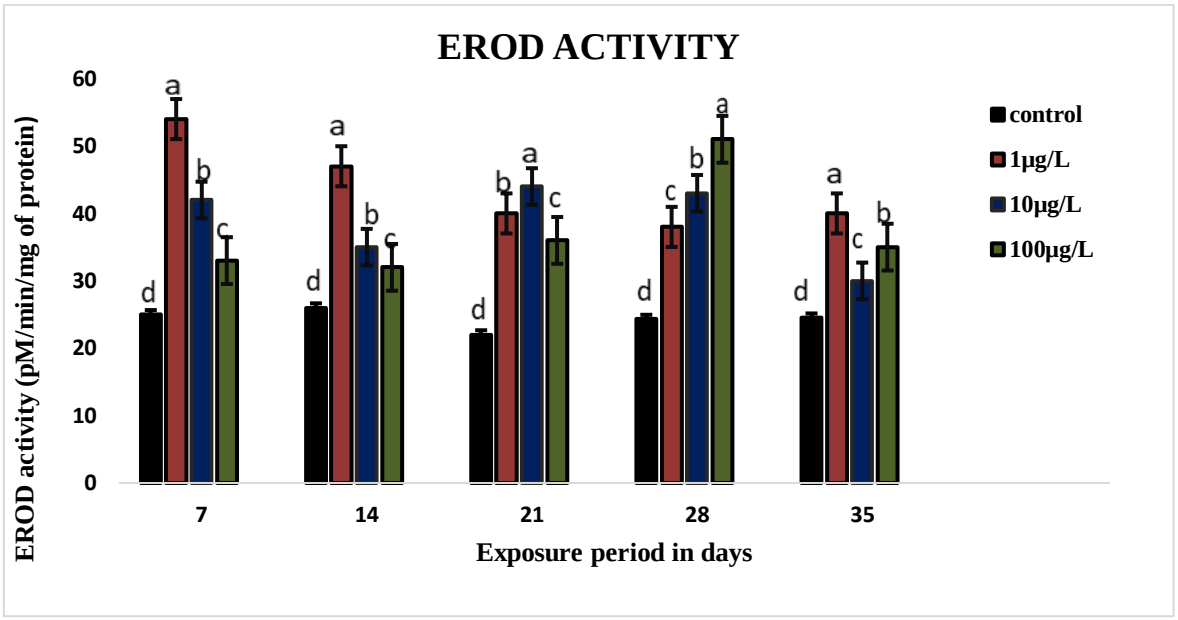


Fig. 3. Changes in hepatic EROD activity in *L. rohita* following sub-chronic exposure to fluoxetine (FLX) for 35 days. Bars represent mean values, and error bars indicate the standard error (SE) of five replicates. Asterisks denote significant differences at $P < 0.05$.

FLX exposure also induced concentration- and time-dependent genotoxic effects in the gills, as evidenced by increased DNA strand breaks (**Fig. 4).** Control fish showed minimal DNA damage throughout, while those exposed to 1 µg/L displayed significant but moderate increases. Fish in the 10 µg/L and 100 µg/L groups exhibited progressively greater DNA damage, with the highest levels recorded at day 35, indicating cumulative genotoxic stress over time.

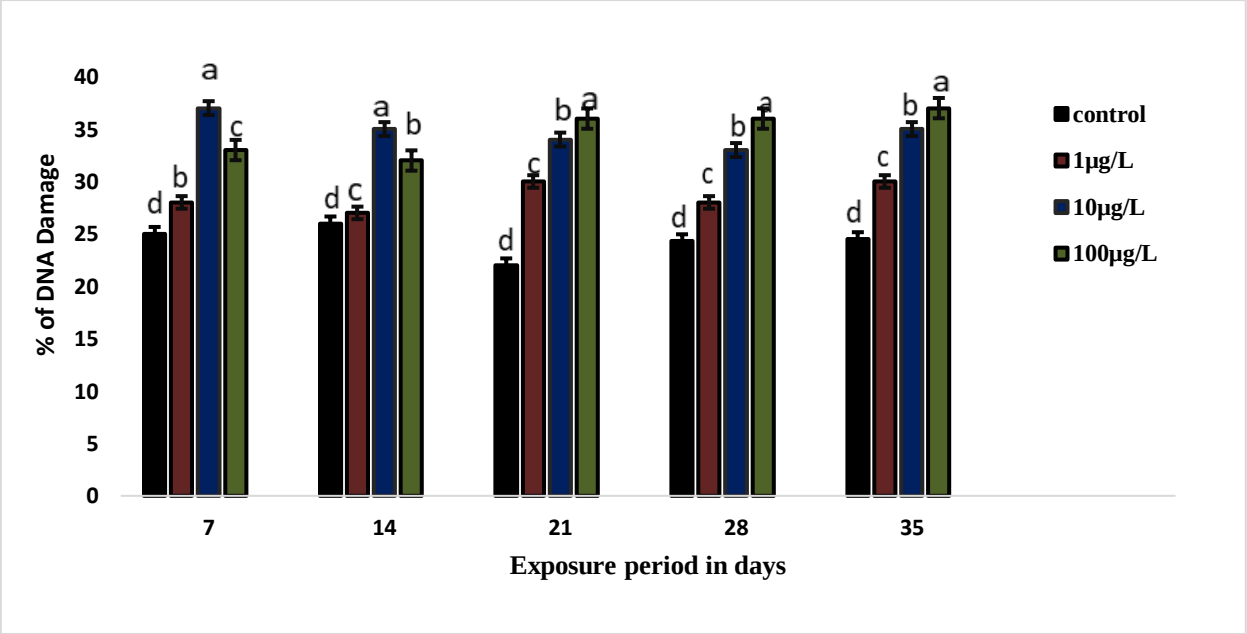


Fig. 4. DNA **damage in gill tissue of *L. rohita* following sub-chronic exposure to fluoxetine (FLX) for 35 days. Bars represent mean values, and error bars indicate the standard error (SE) of five replicates. Significant differences are indicated at $P < 0.05$.**

Overall, the results demonstrate that chronic FLX exposure in *L. rohita* leads to pronounced behavioral suppression, increased movement delay, progressive gill DNA damage, and dose-dependent modulation of hepatic detoxification capacity.

DISCUSSION

Pharmaceutical contaminants, particularly selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (FLX), are increasingly detected in freshwater systems worldwide due to their widespread use and persistence (Richendrfer and Creton, 2017; López-Serna *et al*., 2013; McEachran *et al*., 2018). These compounds can affect non-target organisms by altering behavior, inducing genotoxicity, and disrupting detoxification pathways. In this study, chronic exposure of *L.rohita* to environmentally relevant concentrations of FLX (1, 10, and 100 µg/L) significantly affected behavioral responses, gill DNA integrity, and hepatic EROD activity.

FLX exposure led to reductions in locomotor activity, increased movement latency, and decreased feeding, particularly at higher concentrations. The proportion of actively feeding fish and total activity duration were notably decreased. These behavioral impairments are likely due to FLX interference with serotonergic pathways, which play key roles in regulating appetite and activity in fish. Previous studies have similarly reported decreased feeding behavior and impaired growth in aquatic organisms exposed to FLX (Correia *et al*., 2023).

Our findings are consistent with earlier research showing that FLX modulates serotonergic signaling and suppresses behavior in Gulf toadfish (*Opsanus beta*) (Panlilio *et al*., 2019), alters personality traits in *Neogobius fluviatilis* and *Gobio gobio* (Grzesiuk & Pawelec, 2021), and impairs locomotor activity and induces anti-anxiety responses in *Gambusia holbrooki* at higher concentrations (Meijide *et al*., 2018). Ecologically, reductions in activity and feeding could impair predator avoidance and foraging efficiency, potentially affecting survival and reproductive success (Gerhardt, 2007).Significant DNA strand breaks were observed in gill tissues of FLX-exposed *L. rohita*, indicating genotoxic stress. Gills are directly exposed to waterborne contaminants and are therefore highly sensitive to DNA damage. Similar effects have been reported in other teleosts, including *Argyrosomus regius* and *Oreochromis niloticus*, where FLX exposure increased gill DNA damage (Duarte *et al*., 2020; Farias *et al*., 2019). Such genotoxicity may compromise respiratory function and overall physiological resilience.

The observed increase in hepatic ethoxyresorufin-O-deethylase (EROD) activity indicates that FLX exposure induces cytochrome P450 1A enzymes, reflecting a metabolic response aimed at detoxification. Comparable induction of EROD activity has been reported in the liver of topmouth gudgeon (*Pseudorasbora parva*) (Chen *et al*., 2018), zebrafish at high FLX concentrations (Zindler *et al*., 2020), and in PLHC-1 fish hepatoma cells (Thibaut & Porte, 2008). The persistence of elevated EROD activity after the depuration period suggests prolonged effects of FLX on fish metabolic pathways. Similar non-monotonic patterns have been reported in *Pomatoschistus microps* and *Argyrosomus regius* (Duarte *et al*., 2020). Altered detoxification capacity at higher FLX concentrations may increase vulnerability to other xenobiotics and environmental stressors. Integrating behavioral, genotoxic, and EROD endpoints highlights the multi-level impacts of FLX. Behavioral changes were the most sensitive, detectable even at low concentrations, whereas gill DNA damage reflected cellular-level impairment and EROD activity indicated potential disruptions in hepatic detoxification. Collectively, these effects suggest that chronic FLX exposure may reduce individual fitness and compromise population resilience in freshwater ecosystems.

In conclusion, environmentally relevant concentrations of FLX can impair behavior, induce genotoxic stress, and disrupt detoxification enzyme activity in *L. rohita*. Reduced locomotion and feeding, combined with DNA damage and altered hepatic metabolism, may negatively affect survival and ecological fitness. Given the widespread occurrence of FLX in aquatic environments, further long-term and multigenerational studies are warranted to fully assess its ecological risks (Margiotta-Casaluci *et al*., 2014).

CONCLUSION

In summary, the hepatic biotransformation enzymes of *L.rohita* were stimulated through the administration of FLX, leading to the observation of significant oxidative stress responses. Only higher concentrations (µg/L) induced behavioral and neurological effects. In order to enhance the environmental risk assessment of FLX and other SSRIs (Selective Serotonin Reuptake Inhibitors), further investigation is necessary to obtain additional data regarding the variability of responses among different species, as well as the long-term effects of chronic exposure to environmentally relevant concentrations in non-model organisms.

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