



Analyzing the impact of oral contraceptive pills (I-Pill) on melanophores of some freshwater fish species

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Abstract

Aquatic toxicologists have identified that pharmaceutical effects on chromatophores are reliable pollution indicators. Melanophores, which contribute to skin pigmentation, are influenced by both the nervous and endocrine systems. This study focuses on assessing the effects of an oral contraceptive pill (I-Pill) on melanophores in freshwater fish species: *Cyprinus carpio*, *Catla catla*, and *Labeo rohita*. Findings show that I-Pill disrupts melanophores structure, affecting pigmentation. Three types of melanophores, punctate, stellate, and Reticulostellate, were observed across the species, with their number and shape significantly altering over time with exposure. In *Labeo rohita*, melanophores began to disappear after 96 hours of exposure. The study highlights that I-Pill influences both the surface area and the endocrine system of the fish, with melanophores acting as indicators of stress conditions.

Keywords: *Catla catla*, *Cyprinus carpio* (L.), *Labeo rohita*, Melanophores, I-pill, 96-hour.

Introduction

The rapid expansion of India's pharmaceutical industry has raised the potential for environmental contamination, largely due to the disposal of pharmaceuticals through medical centers and domestic wastewater. Given the physiologically active nature of these drugs, their presence in ecosystems is a concern, particularly for non-target aquatic species such as fish (Fent *et al.*, 2006; Ankley *et al.*, 2007) [2, 3]. Research indicates that even environmentally relevant concentrations of pharmaceuticals can have toxicological effects on aquatic organisms, especially fish. However, studies specifically examining the ecological impact of pharmaceutical residues on aquatic physiology remain limited in India (Saravanan *et al.*, 2011) [11].

Elevated concentrations of various pharmaceutical drugs, including ciprofloxacin, losartan, and cetirizine, have been detected in sewage effluents (Larsson *et al.*, 2007) [6]. Further studies have documented the toxicological effects of specific pharmaceuticals on fish species in India, such as clofibrac acid, diclofenac, and ibuprofen, affecting major Indian carps (Saravanan *et al.*, 2013) [1], and oxytetracycline exposure resulting in significant hematological and enzymological changes in *Labeo rohita* (Ambili *et al.*, 2013) [1]. Recent research has reinforced these concerns, with studies highlighting the inefficiency of conventional wastewater treatment plants in removing pharmaceutical contaminants, leading to their accumulation in surface and groundwater systems (Frontiers in Microbiology, 2022) [4]. This persistence poses risks to aquatic life due to the bioactive nature of these substances.

A review by (Sharma *et al.*, 2017) [13] emphasized the occurrence of pharmaceuticals and personal care products in Indian water bodies, underscoring the need for comprehensive research into their ecological impacts. Moreover, emerging studies have explored bioremediation techniques, such as mycoremediation, which utilizes fungi to degrade pharmaceutical pollutants, presenting a

promising strategy to mitigate environmental risks (Frontiers in Microbiology, 2022) [4].

These findings underscore the urgent need for detailed studies into the sources, pathways, and ecological impacts of pharmaceutical residues in India's aquatic ecosystems, along with the development of effective remediation strategies.

The extensive use of oral contraceptives, particularly those containing the synthetic estrogen 17- α Ethinylestradiol (EE2), has raised environmental concerns due to their incomplete removal during wastewater treatment. While humans absorb approximately 80% of EE2, the remaining 20% is excreted and enters wastewater systems. Conventional treatment methods often fail to eliminate EE2, allowing residual amounts to persist in treated effluents that re-enter aquatic ecosystems. As a result, EE2 contamination has been linked to histological and reproductive abnormalities in aquatic organisms (Fent *et al.*, 2006) [3].

Melanophores, specialized pigment cells responsible for coloration in fish, originate from the neural crest during embryonic development. These cells react to external factors, including neurotransmitters and hormonal fluctuations, which influence pigment dispersion. Research has demonstrated that fish melanophores contain cholinergic receptors that regulate pigment transport (Parker, 1948; Scott, 1965; Miyashita and Fujii, 1973) [7, 8, 12]. Recent findings indicate that endocrine disruptors, such as EE2, can interfere with melanophore function and alter pigmentation patterns, making them potential biomarkers for environmental contamination (Silva *et al.*, 2019; Zhang *et al.*, 2020) [14, 21].

Numerous studies have reported the widespread presence of pharmaceutical contaminants, including EE2, in aquatic environments, with significant consequences for fish populations. The exposure to EE2 has been shown to disrupt endocrine signaling in fish, adversely affecting reproductive and developmental processes, including fertility and growth

(Sun *et al.*, 2018) [17]. Additionally, pharmaceutical pollutants such as EE2, ibuprofen, and diclofenac have been associated with histopathological damage to the liver, kidneys, and gills, indicating the physiological strain of prolonged exposure (Khetan and Collins, 2018).

Despite global efforts to investigate the ecological risks of pharmaceutical pollutants, research in India remains limited, emphasizing the need for localized studies. With increasing pharmaceutical waste discharge in India, particularly in regions with intensive aquaculture, there is an urgent need to examine the effects of contaminants like EE2 on ecologically and commercially important fish species such as *Cyprinus carpio* and *Catla catla*. This study aims to address this knowledge gap by investigating the toxicological impact of I-Pill on melanophore function in these species, which play a vital role in Karnataka's freshwater ecosystems.

Materials and Methods

The study was conducted on three freshwater fish species: *Cyprinus carpio* (L.), *Catla catla*, and *Labeo rohita*. In the experimental setup, water quality parameters were carefully maintained at levels suitable for each species. Fish were exposed to the oral contraceptive I-Pill at a concentration of 4.7 grams/L, selected for its environmental relevance. The exposure was administered over four durations: 24, 48, 72, and 96 hours.

Fish species were procured from the Turvekere Fish Farm in Tumkur District, Karnataka. Using forceps, ten scales were extracted from each fish's dorsolateral region beneath the head, tail, and lateral sides. These scales were placed in separate plastic containers with the I-Pill solution at the designated concentration for the specified time intervals, while control scales were kept in physiological saline. After each exposure period, the scales were rinsed in a saline solution (NaCl: 12.8, KCl: 2.7, CaCl₂: 1.8, Glucose: 5.6, pH 7.4) and mounted on glass slides for observation under a stereo binocular microscope.

The analysis involved quantifying changes in melanophores characteristics, including the number, shape, and types (punctate, stellate, and Reticulostellate). Statistical analysis was performed to compare the differences between the exposed and control groups, evaluating significant alterations in melanophores patterns due to I-Pill exposure.

Results

The scales of *Catla catla*, *Cyprinus carpio* (L.), and *Labeo rohita* exposed to I-Pill (4.7 g/L) showed a decrease in the number of melanophores and alterations in their shape over time.

In *Catla catla* exposed to the I-Pill, melanophores distribution shows distinct changes over time. Only Reticulostellate melanophores are present in the control group, with an average count of 31.2 ± 0.296. After 24 hours of exposure, punctate melanophores appear with a count of 31.2 ± 0.196, stellate melanophores increase to 42.1 ± 0.240, while Reticulostellate melanophores remain at 31.2 ± 0.296. By 48 hours, punctate melanophores decrease to 19.2 ± 0.196, stellate melanophores reduce slightly to 34.3 ± 0.260, and Reticulostellate melanophores also decline to 24.9 ± 1.085. No melanophores are observed at 72 hours; at 96 hours, only Reticulostellate melanophores are present, with a reduced count of 18.0 ± 0.099.

In *Cyprinus carpio* exposed to the I-Pill, notable changes in melanophores types are observed over time. Initially, in the control group, only Reticulostellate melanophores were present with an average count of 53.22 ± 2.587. After 24 hours of exposure, punctate melanophores appear with a count of 24.2 ± 0.970, and stellate melanophores increase to 51.2 ± 2.587, while Reticulostellate melanophores are not detected. At 48 hours, punctate melanophores are absent, stellate melanophores decrease to 35.4 ± 0.787, and Reticulostellate melanophores reappear at 38.5 ± 0.580. After 72 hours, punctate melanophores are again observed at 13.6 ± 3.88, stellate melanophores decline to 21.5 ± 0.580, and Reticulostellate melanophores are absent. By 96 hours, only Reticulostellate melanophores remain, showing a further reduction to 14.9 ± 0.242.

In *Labeo rohita* exposed to the I-Pill, significant changes occur in melanophores types over time. Initially, only Reticulostellate melanophores were present in the control group, with an average count of 42.4 ± 0.040. After 24 hours, punctate melanophores appear with a count of 33.6 ± 3.880, stellate melanophores are observed at 31.2 ± 0.196, and Reticulostellate melanophores decrease to 29.4 ± 0.084. By 48 hours, punctate melanophores further decline to 22.0 ± 3.880, stellate melanophores reduce to 20.9 ± 0.242, and Reticulostellate melanophores drop to 18.8 ± 0.196. At 72 hours, melanophores are absent. After 96 hours, only Reticulostellate melanophores are detected, with a reduced count of 11.9 ± 0.242.

Table 1: Changes in Melanophores of *Catla catla* Exposed to I-Pill

| Exposure Duration | Punctate Melanophores (Count ± SE) | Stellate Melanophores (Count ± SE) | Reticulostellate Melanophores (Count ± SE) |
|-------------------|------------------------------------|------------------------------------|--|
| Control | - | - | 31.2 ± 0.296 |
| 24 hours | 31.2 ± 0.196 | 42.1 ± 0.240 | 31.2 ± 0.296 |
| 48 hours | 19.2 ± 0.196 | 34.3 ± 0.260 | 24.9 ± 1.085 |
| 72 hours | - | - | - |
| 96 hours | - | - | 18.0 ± 0.099 |

Table 2: Changes in Melanophores of *Cyprinus carpio* Exposed to I-Pill

| Exposure Duration | Punctate Melanophores (Count ± SE) | Stellate Melanophores (Count ± SE) | Reticulostellate Melanophores (Count ± SE) |
|-------------------|------------------------------------|------------------------------------|--|
| Control | - | - | 53.22 ± 2.587 |
| 24 hours | 24.2 ± 0.970 | 51.2 ± 2.587 | - |
| 48 hours | - | 35.4 ± 0.787 | 38.5 ± 0.580 |
| 72 hours | 13.6 ± 3.88 | 21.5 ± 0.580 | - |
| 96 hours | - | - | 14.9 ± 0.242 |

Table 3: Changes in Melanophores of *Labeo rohita* Exposed to I-Pill

| Exposure Duration | Punctate Melanophores (Count ± SE) | Stellate Melanophores (Count ± SE) | Reticulostellate Melanophores (Count ± SE) |
|-------------------|------------------------------------|------------------------------------|--|
| Control | - | - | 42.4 ± 0.040 |
| 24 hours | 33.6 ± 3.880 | 31.2 ± 0.196 | 29.4 ± 0.084 |
| 48 hours | 22.0 ± 3.880 | 20.9 ± 0.242 | 18.8 ± 0.196 |
| 72 hours | - | - | - |
| 96 hours | - | - | 11.9 ± 0.242 |

Discussion

Pharmacological research indicates that melanophores contain cholinergic receptors responsible for mediating pigment

dispersion (Parker, 1948; Scott, 1965; Miyashita and Fujii, 1973) [7, 8, 12]. The movement of melanophores, which contributes to pigmentation, is regulated by both the nervous and endocrine systems. Various chemical messengers and neurotransmitters influence the processes of pigment aggregation and dispersion (Sinha *et al.*, 1999) [15]. Several studies have also examined the effects of hormones, chemicals, and pharmaceutical compounds on chromatophores (Yamada *et al.*, 1984; Watanabe *et al.*, 1965; Praveen *et al.*, 1993) [9, 19, 20].

The study found that exposure to the lethal concentration of I-Pill (4.7 g/L) resulted in melanophore dispersion and structural disruptions, which were noticeable in the early hours of exposure. In contrast, the control group exhibited a uniform melanophore arrangement. All three fish species in the study displayed three types of melanophores: punctate, stellate, and reticulostellate, whose numbers progressively declined with prolonged exposure. In *Labeo rohita*, melanophore loss began after 96 hours. These findings suggest that I-Pill induces melanophore alterations, indicating environmental stress due to pharmaceutical contamination. To prevent the indiscriminate release of such drugs into aquatic ecosystems, regulatory measures are necessary. Given that melanophore changes reflect the broader toxicological effects of pharmaceuticals on aquatic life, further research is essential to understand and mitigate these impacts.

Conclusion

In conclusion, the present findings clearly demonstrate that exposure to I-Pill causes marked alterations in melanophore structure and distribution in fish, highlighting its potential as an environmental stressor. The observed dispersion, structural damage, and gradual loss of different melanophore types with increasing exposure duration indicate a disruption of normal pigment regulatory mechanisms governed by the nervous and endocrine systems. The absence of such changes in the control group further confirms that these effects are directly linked to pharmaceutical exposure. Since melanophore responses are sensitive indicators of physiological stress, the alterations recorded in this study reflect the broader toxicological impact of pharmaceutical contaminants on aquatic organisms. These results emphasize the urgent need for stricter regulation and responsible disposal of pharmaceutical products to prevent their entry into aquatic ecosystems. Additionally, melanophore-based biomarkers may serve as valuable tools for early detection of pharmaceutical toxicity, and further studies are essential to better understand long-term ecological consequences and develop effective mitigation strategies.

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